Characterisation of Antimicrobial Usage in Small-Scale Commercial Chicken Farms in the Mekong Delta of Vietnam

Thesis

CHARACTERISATION OF ANTIMICROBIAL USAGE IN SMALL-SCALE COMMERCIAL CHICKEN FARMS IN THE MEKONG DELTA OF VIETNAM

by

Nguyen Van Cuong

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Oxford University Clinical Research Unit

Hospital for Tropical Diseases

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Declaration of the candidate's role in the thesis

I declare that the contents of this thesis are predominantly my own work. The thesis was written under the supervision of my director of studies, Dr. Juan Carrique-Mas, with input of my co-supervisors Dr. Marc Choisy and Dr. Pawin Padungtod.

I reviewed all scientific papers, extracted their content and developed the manuscript for the literature review (Chapter 3) under the supervision of Dr. Juan Carrique-Mas.

Data for the next three chapters (Chapters 4, 5, 6) came from ViParc project where I worked as project coordinator. I contributed to the development of all questionnaires and data collection tools, as well as in the training to field staff for data collection. All field data was collected by field staff affiliated to the Sub-Department of Animal Health Production and Aquaculture of Dong Thap (SDAH-DT) province. The data was checked for consistency and accuracy by the project data manager and by myself before the analyses. Data for next chapter (Chapter 7) came from a cross-sectional survey where data was collected by students with veterinary or biology science background. For the statistical analyses, I am supported by Dr. Juan Carrique-Mas (all chapters) and Dr. Marc Choisy. The support of Dr. Choisy was particularly important in Chapter 6, which required advanced programming skills. I declare that I drafted and developed all manuscripts (either published or submitted to scientific journals) which are the five results chapters of this thesis. All other authors critically contributed to review the content of these manuscripts. This thesis is written by myself after receiving feedback from my supervisors.

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Lời nhắn gửi của "bố"

Lời đầu tiên, chồng muốn nhắn gửi là cho người vợ dấu yêu của mình, chồng cảm ơn vợ yêu đã đồng hành cùng chồng thời gian vừa qua. Khi chồng viết những dòng chữ này, cũng là thời điểm kỉ niệm một năm ngày cưới của vợ chồng mình. Một năm đã qua, vợ đã trải qua nhiều khó khăn, nhưng vợ đã cố gắng rất nhiều, chồng rất vui sướng và hạnh phúc vì điều đó. Chồng cảm ơn vợ vì những hi sinh vợ dành cho chồng và cho gia đình mình.

Những ngày này, cả nhà mình rất vui, khi đã đón thêm một thành viên mới đó là "bé chuột". Vợ đã trở thành mẹ ngay cả khi bé chuột chưa chào đời, một việc mà có lẽ chỉ với một tình thương thực sự, vợ mới có thể đối mặt và vượt qua những khó khăn và thử thách đầy chông gai. Không có lời nào có thể diễn đạt được hạnh phúc của bố, có lẽ nhưng giấc ngủ ngon khi bên vợ là minh chứng rõ nét nhất. "Bé chuột", anh Long, chị Linh sẽ là những thiên thần bé nhỏ của gia đình mình và có lẽ sẽ là những nhà khoa học tương lai! Vợ nhỉ? Chúc "bé chuột" của bố ngoan, chúc anh hai Y long, chị ba Ý Lính luôn đồng hành cùng bố mẹ, luôn học giỏi, khỏe mạnh các con nhé.

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Publication coming from this work

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2. **Cuong NV**, Phu DH, Van NTB, Dinh Truong B, Kiet BT, Hien BV, Thu HTV, Choisy M, Padungtod P, Thwaites G, Carrique-Mas J. ***High-Resolution Monitoring of Antimicrobial Consumption in Vietnamese Small-Scale Chicken Farms Highlights Discrepancies Between Study Metrics***. Front Vet Sci. 2019 Jun 21;6:174. doi: 10.3389/fvets.2019.00174. PMID: 31294033; PMCID: PMC6598194.

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Other publications where I contributed during my PhD studies

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Abstract

Antimicrobial resistance (AMR) is a global threat to the health and wealth of nations. The AMR crisis has been attributed to the overuse and misuse antimicrobials. Excessive use of antimicrobials in animal production is one of the contributing factors to this global threat. This thesis aims to characterize antimicrobial used (AMU) in small-scale chicken farms in the Mekong Delta region of Vietnam. This includes consumption of antimicrobials mixed with water by the farmer as well those included in commercial feeds as antimicrobial growth promoters (AGPs). The epidemiological data gathered is used to investigate the relationship between AMU and disease.

First, I conducted a systematic literature review to provide an overview of metrics and methodologies used to measure AMU in animal production in the scientific literature, as well as reviewing existing data on AMU in different species in order to identify data gaps worldwide. Then, I performed a longitudinal study on a large cohort of small-scale chicken farms in Dong Thap province in the Mekong Delta region of Vietnam from October 2016 to May 2018 to investigate in detail the types and amounts of antimicrobials consumed, as well as the relationship between AMU and disease. On average, chickens consumed antimicrobials mixed in water over 382.6 per 1,000 days, or 323.4 mg (SEM ±11.3mg) per kg of chicken produced. The average amounts of AAIs in commercial feed given to produce one kg of chicken was 84.8mg (SEM ±9.3mg). Prophylactic AMU did not reduce the probability of disease, and administration of some antimicrobial classes did increase the risk of disease. Therapeutic AMU often had an effect on mortality but the pattern was inconsistent across the combinations of antimicrobial classes and clinical signs. Thirdly, I performed a study in mixed-species small-scale farms typical of the Mekong Delta in order to investigate whether AMU

data could be gathered using a simple cross-sectional study design. Results highlight the disproportionately high levels of AMU in animal production in the Mekong Delta region, and provide a guideline for the estimation of AMU from simple cross-sectional surveys on farms

Results from this thesis suggest that efforts to promote responsible use of antimicrobials and limit excessive AMU should primarily target animal production. The message 'prophylactic AMU does not overall reduced the probability of disease in flocks' should be further disseminated to poultry farming communities.

Abbreviations

AAI	Antimicrobial Active Ingredient
ADD	Animal Daily Dose
AGP	Antimicrobial Growth Promoter
AMR	Antimicrobial Resistance
AMU	Antimicrobial Usage
ARG	Antimicrobial Resistance Encoding Gene
CIA	Critical Importance Antimicrobial
DDD	Defined Daily Dose
EEA	European Economic Area
EMA	European Medicine Agency
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
EU	European Union
FAO	Food And Agriculture Organization Of The United Nations
GAP	Global Action Plan
GLASS	Global Antimicrobial Resistance and Use Surveillance System
HGT	Horizontal Gene Tranfer
HICs	High-Income Countries
JACS	Japan Antimicrobial Consumption Surveillance
MARD	Ministry of Agriculture and Rural Development
OIE	World Organisation for Animal Health
PCU	Population Correction Unit
WAHIS	OIE World Animal Health Information System
WHO	World Health Organisation

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Chapter 1

Introduction

1.1 The global issue of antimicrobial resistance

Antimicrobial resistance (AMR) has been defined as a global emergency (O'Neill 2015a; WHO 2015). This phenomenon often occurs when microorganisms are exposed to antimicrobial agents (Prestinaci 2015). Since the discovery of the first antibacterial antimicrobial over nearly a century ago, virtually all infection-causing microorganisms, have, in varying degrees, developed resistance to any new antimicrobials released (Gaynes 2017). The AMR health crisis has been attributed to the overuse and misuse of antimicrobials (Ventola 2015). The situation has been worsening since the number of resistance organisms is mounting and many have developed resistance to multiple antimicrobials (Levy and Marshall 2004). This increase in AMR has been reported not only in hospitals but also in community and animal production (i.e. farms) settings. Since antimicrobial resistance-encoding genes (ARG) can be transferred between different organisms, the AMR situation in humans is interlinked with that of animals and the environment (Woolhouse, Ward et al. 2015). Excessive antimicrobial use (AMU) is the major factor driving AMR (FAO 2016). In addition, other factors such as high human and animal population densities, inefficient hygiene/biosecurity in farming practices or hygiene/infectious disease control practices, spillage of sewage/farm manure control and bacterial contamination of food may also contribute to the spread of AMR (Holmes, Moore et al. 2016; Robert Davies 2015). The emergence and spread of drug-resistant pathogens further enhanced by economic booming and international travel, continues to threaten our ability to treat common infections (Frost, Van Boeckel et al. 2019; Jasovsky, Littmann et al. 2016).

AMR affects all countries, but its burden on low- and middle-income countries (LMICs) is disproportionately high (Pokharel, Raut et al. 2019). LMICs are particularly

vulnerable because they generally lack adequate healthcare (including animal veterinary) systems (Semret and Haraoui 2019). In these countries, farming systems are typically small-scale, and often used antimicrobial extensively, while practicing poor standards of biosecurity and disease control (Van Boeckel, Pires et al. 2019). The Southeast Asia region has a high incidence of infectious diseases and AMR, and is regarded as a hotspot (Zellweger, Carrique-Mas et al. 2017). Data on phenotypic resistance in commensal *E. coli* from Vietnam and Thailand have shown an increase in prevalence of AMR over the years (Nhung, Cuong et al. 2016). AMR is not bound by country borders, and may affect multiple geographic locations, becoming therefore a global threat to the health and wealth of all nations (O'Neill 2015a). AMR has been identified as the top priority for global health action (Chioro, Coll-Seck et al. 2015). Similar to climate change, global AMR is a shared global concern that requires local solutions. Tackling AMR at national and local levels can produce direct benefits and improve the situation on a global scale (Gelband and Laxminarayan 2015).

1.2 Mechanisms of generation and transmission of AMR

Since the first introduction of antimicrobials, microorganisms have gradually developed resistance over time (Reygaert 2018). These resistance mechanisms may be native to the microorganisms, or may be acquired. Acquired resistance may be the result of gene mutations or 'horizontal gene transfer' (HGT) (i.e. ARGs that are transferable among microorganisms through 'transformation', 'transposition' or 'conjugation'). Four main mechanisms of resistance have been identified, including: (1) limiting uptake of an antimicrobial, (2) modification of its cell target, (3) inactivation, and (4) active efflux of antimicrobial drugs (Reygaert 2018).

The transmission of AMR bacteria and genes across systems can occur through complex pathways that may include humans and animals and the environment (Graham, Bergeron et al. 2019; Woolhouse, Ward et al. 2015; Da Costa, Loureiro et al. 2013). A number of bacterial (pathogenic and/or commensals) species and ARGs (plasmid, mobiles genetics elements) may be exchanged between humans (hospital and community), animals (farm, domestic, wildlife and aquatic animals), and the environment (Baquero, Coque et al. 2019). Resistant organisms may be transmitted between animal, between humans or across species through direct (i.e. exposure to infected individuals/animals, handling and consumption of contaminated food,) or indirect (i.e. through environment/water contaminated with untreated human waste/animal manure) contact.

1.3 Use of antimicrobials in animal production

1.3.1 Purposes of AMU in animal production

Since their discovery and introduction during the earlier part of the previous century, antimicrobials have yielded considerable improvements in human and veterinary medicine. In animal production settings (including terrestrial and aquatic animal farms, feed mills); antimicrobials are used for four main purposes: (1) to increase feed conversion ratio (i.e. to make animals grow faster with less feed) (i.e. 'antimicrobial growth promotion'); (2) to prevent disease occurring in healthy animals ('prophylactic' use), and (3) to treat flocks/herds before experiencing any level of disease onset or 'metaphylactic' and finally to cure infectious disease or 'therapeutic' (Kirchhelle 2018; Pagel and Gautier 2012). Antimicrobials added to food rations in low concetrations with the aim of increasing the grow of animal and feed conversion efficiency are called

'antimicrobial growth promoters' (AGPs) (Butaye, Devriese et al. 2003), although their mechanism of action remain unknown (Dibner and Richards 2005). Also, antimicrobials at sub therapeutic doses are often used prophylactically in certain animal production settings (Landers, Cohen et al. 2012). Metaphylactic use is defined as the mass treatment of animal population currently not experiencing disease, but at risk of disease as observed in contact animals. There are some differences between AMU in humans and food animals, particularly with regards to AMU for 'growth promotion' and 'prophylactic' purposes. The former is not practiced in human medicine, and the latter only takes place in the context of surgical procedures or in the case of high risk of exposure to an infectious agent (Prescott 2017). In humans, administration of antimicrobials is overwhelmingly therapeutic, with doses usually based on age (less frequent on body weight) (Pagel and Gautier 2012).

1.3.2 AMU in poultry production

Poultry meat is one of the most common food commodities worldwide. Antimicrobials play a positive role in poultry production since they help control disease and may contribute to improve the flock performance (Christian Agyare 2018). An estimate from the literature showed that, in term of doses, poultry is the target of the highest amount of antimicrobials worldwide (138 doses per 1,000 animal-days. The most used antimicrobial classes used in poultry are tetracyclines, followed by macrolides, polypeptides and penicillins (Cuong, Padungtod et al. 2018). There are knowledge gaps regarding how AMU are used in industry scale production compared with small-scale flocks. Antimicrobial agents are often administered at flock level through via drinking water or feed, depending on the objective of the administration. Because it enables large numbers of birds to be treated conveniently and cheaply at the same time. Drinking

water is the preferred mode of therapeutic or metaphylactic administration, because diseased birds usually tend to stop eating but will often continue to drink. An alternative to the drinking water, is the administration of a drug through the feed, often is the preferred mode of prophylactic or AGP administration (Landoni and Albarellos 2015).

1.3.3 Classification of antimicrobials according to WHO and OIE

The WHO has defined three categories of antimicrobials based on two criteria: (i) Whether the antimicrobial is the sole therapy or one of few alternatives to treat serious human disease; and (ii) whether the antimicrobial is used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases causes by organisms that may acquire resistance genes from non-human sources. The three categories defined are: (1) Critically important antimicrobials (criteria 1 and 2), (2) Highly important antimicrobials (criteria 1 or 2) and (3) Important antimicrobials (neither criteria 1 nor 2) (FAO 2007) .

The OIE has defined three different categories of antimicrobials based on two criteria: (i) Whether the response rate to the questionnaire regarding Veterinary Critically Important Antimicrobials was met (more than 50% of the respondents identified the importance of the antimicrobial class in their response to the questionnaire) and (ii) whether the antimicrobial were identified as essential against specific infections and there was a lack of sufficient therapeutic alternatives. The three categories defined are: (1) Veterinary critically important antimicrobials (criteria 1 and 2), (2) Veterinary highly important antimicrobials (criteria 1 or 2) and (3) Veterinary important antimicrobials (neither criteria 1 nor 2) (FAO 2007).

The major different between WHO and OIE was in the critically important antimicrobials (CIA) category. A number of antimicrobials classes appear only in the

WHO list included: carbapenems, ansamycins, glycopeptides, streptogramins and oxazolidinones; whereas phenicols, sulfonamides and diaminopyrimidines, and tetracyclines was considered only as critically important for animal health by OIE (FAO 2008).

There are several different points that only existed in WHO CIA which included: (1) only glycylcycline of tetracyclines class is categorized as critically important antimicrobial, the other class members are categorized as highly important antimicrobials, (2) the cephalosporins were separated into two different groups; 1st/2nd generation cephalosporins and 3rd/4th generation cephalosporins, (3) quinolones and fluoroquinolones were grouped together in the same class.

Many antimicrobials used in food-producing animals are identical, or closely related, to antimicrobials used in humans. The WHO and OIE lists demonstrated that critically important antimicrobials are needed in both human and food animal therapy. To mitigate the adverse human health consequences of use of CIA in food-producing animals. A guideline on use of CIA in food-producing animals, recommending that farmers and the food industry stop using antibiotics routinely to promote growth and prevent disease in healthy animals. This guideline aim to help preserve the effectiveness of antibiotics that are important for human medicine by reducing their use in animals (WHO 2017).

1.3.4 Impact of AMU in animal production on the global burden of AMR

There is a strong scientific consensus that AMU in animal production is a substantial driver of AMR in animal populations (O'Neill 2015b). This has been firmly established from on-farm observational studies (Burow, Simoneit et al. 2014; Simoneit, Burow et al. 2015), from country AMU/AMR surveillance data (Asai, Kojima et al. 2005;

Chantziaras, Boyen et al. 2014), as well from meta-analyses of published data (Bell, Schellevis et al. 2014; Tang, Caffrey et al. 2017). There is also a large body of evidence of an association between AMU in food animals and the emergence of AMR in humans, although this type of evidence often based on case studies (Landers, Cohen et al. 2012; Marshall and Levy 2011). Demonstration of this association is difficult probably a reflection of the diversity of ARG and the complexity of AMR transmission mechanisms, which would require very costly longitudinal study. However, there is a consensus that AMR is a One Health issue, and this has provided much of the recent impetus for monitoring and reducing AMU in animal production.

1.3.5 Impact of AMR on disease control and farm productivity

The presence of AMR in animal pathogens are likely to have considerable (negative) impact on livestock health and productivity by reducing the capacity of treating sick animals (FAO 2016; Bengtsson and Greko 2014). A recent study showed that the administration of antimicrobials in the context of small-scale chicken flocks in the Mekong Delta (Vietnam) is likely to result in unsuccessful treatment of disease (i.e. 'treatment failure') in a large fraction of cases. This is because often antimicrobials are used in the absence of confirmatory diagnosis, and are likely to be used in situations of resistant bacterial (Yen, Nhung et al. 2020) or viral infections (Choisy, Van Cuong et al. 2019).

As a result of excessive AMU, bacterial population in animal settings will, to a variable degree, acquire AMR traits, and this may eventually resulted in reducing the effectiveness of antimicrobials, and thus the loss of the necessary armory required for infection control (Landers, Cohen et al. 2012).

1.4 Surveillance systems of AMU in animal production

1.4.1 International surveillance systems

The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), launched by the European Medicine Agency (EMA) in September 2009 was the first multi-country surveillance system on AMU in animal production. ESVAC presented a harmonised approach for the collection and reporting of data on the use of antimicrobial agents in animals in EU and European Economic Area (EEA) Member States (EMA 2009). Antimicrobials used/sold in each country is related to animal populations using the mg/PCU AMU metric:

$$\text{AMU (mg/PCU)} = \frac{antimicrobial\ agents\ reported\ (mg)}{Population\ Correction\ Unit}$$

The numerator corresponds to the sales data corresponding to each antimicrobial active ingredient (AAI), the denominator corresponds to the animal population at country level, expressed as 'Population Correction Unit' (PCU). One PCU is equivalent to 1 kg of animal treated. The total number of PCUs per country, year and animal category are calculated by multiplying the numbers of the various animal categories (number of animals slaughtered, number of livestock, number of animal imported/exported) by their average weight at treatment, estimated as a consensus value for each species across the EU. The average weight values at treatment used to calculate the PCU is given in Table 3, Appendix 2 of the 1st ESVAC report (EMA 2011). Currently the ESVAC report gives overall estimates for all animal species combined (excluding domestic animals). In most EU countries, data on AMU is provided by the industry, and this sales information is combined to animal production statistics (expressed as PCU). EMA has also established standardised units of measurement for reporting antimicrobial

consumption in specific animal species, called the 'defined daily dose' and 'defined course dose' for animals. This metric is expected to be used in future reports of ESVAC, alongside mg/PCU (EMA 2020). In July 2018, EMA launched a project for stratifying sales data of veterinary antimicrobials by animal species, by allocating a proportion of the total sales. This project has piloted in six EU member states to investigate whether this approach may be used in the future for the whole of the EU. The latest ESVAC report includes AMU data on veterinary antibiotics collected from the 31 countries of the ESVAC network. The consumption data excludes companion animals. An overall decrease of 34.6% (from 161.4 down to 105.6 mg/PCU) in sales were observed for the 25 countries that reported sales data to ESVAC from 2011 to 2018. A decrease in sales of all antimicrobial classes has been observed except for aminoglycosides, amphenicols and lincosamides (EMA 2020).

The OIE has taken the lead by creating a global database on the use of antimicrobial agents in animals, in the framework of the Global Action Plan on Antimicrobial Resistance (OIE 2016). In its fourth OIE annual report (2020) on the use of antimicrobial agents intended for use in animals, data on AMU during 2018 was submitted by 153/184 (84%) OIE member countries, but only 118 (64%) countries were able to provide quantitative data on sales of antimicrobial agents. By OIE region, the proportion of countries responded to the survey was highest in America (94%) and Europe (91%) and lowest in Africa (81%), Asia-Far East-Oceania (78%) and Middle East (50%). The fundamental barriers to collect AMU data were lack of regulator frameworks for veterinary products and lack of IT tools, funds and human resources (OIE 2020).

In the OIE report, quantitative sales data of antimicrobials is adjusted for 'animal biomass' using following metric:

$$\text{AMU (mg/kg)} = \frac{antimicrobial\ agents\ reported\ (mg)}{animal\ biomass\ (kg)}$$

The quantities used in animals are grouped into 'all animal species', 'companion animals', 'all food-producing animals', 'terrestrial food-producing animals', and 'aquatic food-producing animals'. Unlike the EU 'PCU', the OIE animal 'biomass', is calculated as the total weight of any live domestic animals living or having been raised over a year in a specific area. It is used as a proxy to represent those likely exposed to the quantities of antimicrobial agents reported. This methodology of calculating animal biomass was developed by the OIE using globally available datasets such as the OIE World Animal Health Information System (WAHIS) and the United Nations Food and Agriculture Organization Statistics (FAOSTAT) (Góchez, Raicek et al. 2019). By OIE region, the quantities adjusted by animal biomass of countries responded to the survey in 2016 was highest in Asia-Far East-Oceania (240.5mg/kg), followed by the America (138.0mg/kg), Europe (68.5mg/kg) and lowest in Africa (45.2mg/kg). Although the report provided global estimates for 2104 and 2015, these results should not be compared and should be interpreted with caution due to the identification of errors of those early reports.

1.4.2 Country-specific AMU surveillance systems

Countries that are able to provide antimicrobial quantity data for either ESVAC or the OIE reports normally have their own in-country data collection mechanisms, although few LMICs have fully developed AMU surveillance systems. Japan established its Antimicrobial Consumption Surveillance (JACS) in 2019. The sales volume of antimicrobials was collect from each marketing authorization holder of veterinary drugs, using a designated reporting form. (Laboratory 2019). Thailand is the only country in the SE Asian region that annually publishes comprehensive AMU data in animals and humans The second report of consumption of antimicrobial agent in Thailand in 2018 was issued in 2020. This report provided baseline data on antimicrobial consumption in human**s** and animals. On average, Thai people consumed 74.4 Defined Daily Doses (DDD)/1,000 inhabitants-days in 2018

(increased 8.8% compared to 2017 data). The most common antimicrobial classes consumed were beta-lactams, penicillins and tetracyclines. Nearly 65% of total antimicrobial consumption belongs to WHO Critical Importance Antimicrobial (CIA) classes**.** The overall antimicrobial consumption among food-producing animal in 2018 was 522.1mg/$PCU_{Thailand}$ (a 6.4% reduction compared to 2017). Penicillins and tetracyclines were the most antimicrobial classes consumed. However, the consumption of CIA has increased compared to 2017 data. For the highest priority group of CIA, macrolides were consumed the most, followed by polypeptides and quinolones.
(Thailand 2020).

1.4.3 Challenges for monitoring AMU

A major challenge for monitoring AMU countrywide is the diversity and rate of change of production systems. Surveillance of AMU in animal production may address different aims: to monitor AMU over time; to compare between different populations; as a benchmark to promote reductions of AMU; and to investigate the association between AMU and AMR. However, the diversity of metrics used in different studies and surveillance systems presents an additional challenge to the comparability of the data (Collineau, Belloc et al. 2017). Recent study review intensively 38 active farm-level AMU monitoring systems from 16 countries showed that these systems differ in many ways, including which data are collected, the type of analyses conducted and their respective output (Sanders, Vanderhaeghen et al. 2020).

As a part of this thesis, I reviewed antimicrobial usage in animal production. The review summarized the methodologies and metrics that available in the literature. Result of this study was included in Chapter 3.

1.5 International efforts aimed at curbing excessive AMU in animal production

A number of global, regional and country-level initiatives aimed at promoting responsible AMU whilst curbing excessive AMU in animal production have been implemented in recent years (Carrique-Mas and Rushton 2017; Health 2016; Nations 2016; Postma, Stark et al. 2015; 'RUMA sets out AMR strategy action plan' 2014). Reduction of unnecessary AMU is considered imperative if the global aim of preserving the efficacy of existing antimicrobials is to be achieved. Large efforts to reduce AMU in livestock in Denmark and Sweden started in the early 1990s through restriction of AGPs in feeds. AGPs have been banned since 2006 across all of the European Union (EU) (Castanon 2007). In the USA, voluntary phasing out of certain AGPs have been implemented since 2013 (FDA 2013). In the Asia-Pacific region, several countries have implemented a full or partial bans of AGP in animal feeds in Korea (2011), Australia (2013) (Laxminarayan 2015), Thailand (2015) (Thamlikitkul, Rattanaumpawan et al. 2015), China (2016) (Walsh and Wu 2016), Vietnam (2018) (Development) 2017) and India (2019) (MOH-FW 2109). In 2018, the EU Parliament approved legislation that came into force in 2020 including a full ban on prophylactic use of antimicrobials in livestock and aquaculture production (Anon. 2020).

1.6 Small-scale chicken production in the Mekong Delta of Vietnam

In LMICs, poultry meat is the most consumed protein commodity because its relatively low capital investment and production costs, as well as the lack of religious objections to its consumption (Anon. 2013). Worldwide annual consumption of animal protein, poultry meat (2018) stands at 33%, second only to pork (40%) (Checkoff 2018). According to the Vietnamese official statistics (2019), of a census of 382M chickens, only 26.1% corresponded to chickens raised in industrial systems (Anon. 2019a), the remainder being raised in backyard and small-scale (semi-intensive) commercial farms.

The Mekong Delta, consists of 13 provinces, located at the southernmost part of Vietnam with a total natural area of 40,000 square km, accounting for 12.2% of the country's natural area, is home to more than 17 million people. The climate in this region is influenced by monsoons with dry (from December to April) and wet (from May to November) season. In the wet season, large areas of the Delta are flooded. In the dry season, water volumes on the Mekong decrease, leading to saline intrusion and the salinization of waterways in the lower Delta. The economy of the Mekong Delta is dominated by agriculture, producing more than 50% of the country's rice. Other agricultural activities such as fruit, vegetable and animals farming, also play an important role (GIZ 2015).

In the Mekong Delta region in 2019 of 82M poultry heads, 63.4% corresponded to chickens (Anon. 2019b), the remaining being mostly ducks, Muscovy ducks and quails. The majority of chicken flocks in this area consist of native breeds, of slow growth flocks over long period (~ 18 weeks) (Carrique-Mas, Van et al. 2019). Raising poultry in mixed species small-scale farms (often including pigs and other species) is very common in this region. This type of farming practice enable farmers to produce enough meat and eggs for household consumption and obtain an income, in addition to other key activities such as rice and aquaculture (Delabouglise, Nguyen-Van-Yen et al. 2019). This region has a large number (est. in 1.5 million) 'market seasonal farms'. These farms only raising flocks during certain times of the year, often targeting specific festivities, notably the Vietnamese New Lunar Year ('Tet')'. These flocks often experienced high disease and mortality risks (Delabouglise, Nguyen-Van-Yen et al. 2019; Carrique-Mas, Van et al. 2019). High amount of AMU was reported in chicken production in this area. However, the existing information on AMU comes from cross-

sectional study designs are often bias by limited record keeping practiced by many such farmers (Carrique-Mas, Bryant et al. 2014; Nguyen, Nguyen et al. 2016). In addition, information gathered form those studies often provide the number of active ingredient used with the total amount used only. When and how antimicrobials were used in those studies are not often described adequately.

1.7 Research questions, aims and objectives of this thesis

This thesis aims to characterize AMU in small-scale chicken farming systems in the Mekong Delta region of Vietnam. The research questions of this study are:

1. What and how antimicrobials (both in water and in feed) are used in chicken flocks raised in in small-scale farming conditions in the Mekong Delta.
2. How AMU affects disease and mortality in flocks raised in these systems.
3. How to measure and compare of AMU in humans and animals in small-scale farming settings in the Mekong Delta of Vietnam.

To address these research questions above, I have conducted five studies to achieve specific objectives including:

1. A literature review to summarize metrics and methodologies used for quantitatively assess AMU in animal production systems.
2. A longitudinal study to describe the types, quantities and critical time points when antimicrobials are used in water using different metrics.
3. A descriptive study to review the labels and to calculate the amounts of antimicrobial active ingredients (AAIs) in commercial feeds.
4. To investigate the impact of prophylactic and therapeutic AMU on flock disease using longitudinal epidemiological data gathered on AMU.
5. A cross-sectional study to measure and compare AMU in humans and animals using different metrics, and extrapolate these magnitudes for the Mekong Delta region of Vietnam.

   These five studies are written as five result chapters (Chapters 3 to 7) and are presented in as scientific manuscripts.

Chapter 2

Materials and Methods

2.1 Literature review

The 'ISI Web of Knowledge' engine (Clarivate Analytics, Philadelphia, PA, USA) was used to search for original scientific articles published in English over the period January 1998 to April 2018. I summarized data from 89 scientific studies reporting AMU data in animal production published in English since 1998. Publications not reporting original research data, or written in languages other than English, were further excluded. Detail of the literature review was presented in Chapter 3.

2.2 Longitudinal study

Data for chapters 4, 5 and 6 came from ViParc project. The ViParc project (acronym for "Vietnamese Platform for Antimicrobial Reduction in Chicken production" , is one of the field-based intervention trials, aiming to reduce antimicrobial usage in food animal systems in the Southeast Asian region, funded by the Wellcome Trust (Carrique-Mas and Rushton 2017). The project targets the small poultry producers. It recruited and followed-up of 120 randomly selected meat chicken farms in Dong Thap province, in the Mekong Delta region of Vietnam. These farms represented about one fourth of the total number of chicken farms (459 farms) that raised for commercial purpose in the two districts (Cao Lanh and Thap Muoi) registered in the farm census. However, this information is impossible to know since the selection was based on the existing census, which is only updated every 4-5 years. The project delivered in two phases, a "baseline" phase (12 months), followed by an intervention phase (18 months). During the 'baseline phase', farmers are required to keep a project log-book where all relevant data (antimicrobial and feed consumption, disease, mortality, vaccination, etc.) is weekly annotated. Farmers are asked to keep containers of all medicines and commercial feed products used. The 'baseline' phase data are used for the analysis of

chapter 4. During the 'intervention' phase, randomly selected farms were allocated into three intervention groups including two intervention groups with different level of veterinary supported and a control group. The 'baseline' and 'intervention' phase data are used for the analysis of Chapters 5 and 6. The details of relevant data are presented separately in each chapter.

2.3 Cross-sectional study

A cross-sectional survey of poultry-raising households in five of 12 districts of Dong Thap province (Mekong Delta, Vietnam) was conducted. The five districts were chosen based on convenience criteria. The chosen criterion were farms that located less than 30km from the provincial capital. We aimed to sample ~100 households. In each selected household, the person identified as being responsible for taking care about family members and animals the most were interviewed. The data collected, based on frequency (days) of antimicrobial consumption, was used to calculate AMU, both in terms of doses and quantities (weight of AAI) for the province and the Mekong Delta region by species. All visits were conducted by affiliated staff of the Sub-Department of Animal Health and Production of Dong Thap (SDAHP-DT) during July 2019.

2.4 Statistical methods

All analyses and figures of this thesis were carried out using R statistical software (www.r-project.org). Chapters 3-7 included intensively descriptive statistic on observational data. A generalized logistic model was used in chapter 4 and a logistic generalized additive model was used in chapter 6.

2.5 Ethics

The ViParc project has been granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Minimal Risk) (Ref. 5121/16). Ethics approval for the cross-sectional study (chapter 7) was obtained from the Oxford Tropical Research Ethics Committee 189 (OxTREC), Oxford, UK (Reference No. 533-19).

Chapter 3

Antimicrobial Usage in Animal Production: A Review of the Literature with a Focus on Low- and Middle-Income Countries

antibiotics

MDPI

Review

Antimicrobial Usage in Animal Production: A Review of the Literature with a Focus on Low- and Middle-Income Countries

Nguyen V. Cuong [1], Pawin Padungtod [2], Guy Thwaites [1,3] and Juan J. Carrique-Mas [1,3,*]

[1] Oxford University Clinical Research Unit, 764 Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam; cuongnv@oucru.org (N.V.C.); gthwaites@oucru.org (G.T.)
[2] Emergency Center for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations, Green One UN House Building, 304 Kim Ma, Hanoi, Vietnam; Pawin.Padungtod@fao.org
[3] Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, Oxford University, Old Road Campus, Headington, Oxford OX3 7BN, UK
* Correspondence: jcarrique-mas@oucru.org

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Abstract: Antimicrobial use (AMU) in animal production is a key contributor to antimicrobial resistance (AMR) worldwide. As consumption of animal protein and associated animal production is forecast to increase markedly over coming years in low- and middle-income countries (LMICs), accurate monitoring of AMU has become imperative. We summarized data from 89 scientific studies reporting AMU data in animal production published in English since 1998, identified through the 'ISI Web of Knowledge' search engine. The aims were as follows: (a) to describe methodologies and metrics used to quantify AMU; (b) to summarize qualitative (on-farm prevalence of use) and quantitative (amounts of antimicrobial active principle) data, in order to identify food animal species at the highest risk of AMU; and (c) to highlight data gaps from LMICs. Only 17/89 (19.1%) studies were conducted in LMICs. Sixty (67.3%) reported quantitative data use, with 'daily doses per animal-time' being the most common metric. AMU was greatest in chickens (138 doses/1000 animal-days [inter quartile range (IQR) 91.1–438.3]), followed by swine (40.2 [IQR 8.5–120.4]), and dairy cattle (10.0 [IQR 5.5–13.6]). However, per kg of meat produced, AMU was highest in swine, followed by chickens and cattle. Our review highlights a large deficit of data from LMICs, and provides a reference for comparison with further surveillance and research initiatives aiming to reduce AMU in animal production globally.

Keywords: antimicrobial use; livestock; poultry; metrics; pigs; cattle; chickens

1. Introduction

Antimicrobials are used worldwide both in humans and in animals for the prevention and treatment of infectious diseases [1]. In addition, in some countries, antimicrobials are used in animal farming as growth promoters [2]. A correlation between antimicrobial use (AMU) and antimicrobial resistance (AMR) in animal production has been firmly established from observational studies [3,4], country AMU/AMR surveillance data [5,6], and statistical meta-analyses [7]. Increased levels of AMR have a negative impact on livestock production, either by reducing farm productivity, or by higher costs of disease treatment [8]. However, much of the impetus for monitoring AMU/AMR in animal production has stemmed from an emerging scientific consensus supporting the contribution of AMU/AMR in animal production on the overall burden of AMR in humans [9–11]. As a consequence of this, a number of global, regional, and national initiatives have recently been implemented to promote responsible use of antimicrobials and to curb excessive AMU in animal production [12–16].

In the European Union (EU), a supranational system to monitor AMU in both humans and animals across EU member states has become a reality [17]. A 2014 joint European Centre for Disease Control/European Food Safety Agency/European Medicines Agency surveillance report estimated that, across 28 EU member states, 8927 tonnes of antimicrobial active ingredients were used for animals, compared with 3821 tonnes used for medical purposes [18]. In the USA, antimicrobials used in food animal production accounted for 70% of total antimicrobial consumption in 2014 [10].

The World Health Organization has projected a global increase in meat production from 218 million tonnes in 1999 to 376 million tonnes in 2030, with relatively greater increases in developing countries [19]. The amounts of antimicrobials aimed at animal production worldwide have been forecast to increase by 67% from 2010 to 2030, mostly driven by increased demand for animal protein and intensification of farming systems in low- and middle-income countries (LMICs) [20], although there is considerable uncertainty around the magnitude of this increase. Very little is known about what food animal species are the target of highest levels of AMU in LMICs, while data from high-income countries (HICs) are far from comprehensive. Because of this, international technical agencies have set up initiatives aimed at monitoring AMU/AMR in animal production with a focus on LMICs [21,22].

Measuring AMU in animal production may address different objectives: monitoring AMU over time, setting benchmarks to promote AMU reductions, and investigating associations between AMU and AMR. However, because AMU can be measured using a large diversity of metrics, posing a considerable difficulty to the comparability of data across studies [17]. In addition, limitations in resources and research capacity typical of many LMIC countries represent an additional challenge [23].

In this article, we reviewed and summarized peer-reviewed original research on AMU in terrestrial food animal production worldwide. The aims were as follows: (1) to document methodologies and metrics used to quantify AMU; and (2) to compile qualitative (i.e., prevalence of usage of specific antimicrobials and antimicrobial classes) and quantitative (amounts of antimicrobial active principle), identifying those food animal species (pigs, poultry, or cattle) at highest risk of AMU. We extracted all raw data and metrics reported in these studies, discussed the limitations of the methodologies used, and documented data gaps in LMICs. We hope that this review helps to encourage further harmonization of methodologies aiming at measuring AMU and achieving AMU reductions in animal production globally.

2. Materials and Methods

2.1. Article Selection

The 'ISI Web of Knowledge' engine (Clarivate Analytics, Philadelphia, PA, USA) [24] was used to search for original scientific articles published in English over the period January 1998 to April 2018. The following terms were used to search publications with titles using the following keywords: (antimicrobial* OR antibiotic*) AND (use* OR usage* OR consumption* OR amount* OR quantity*) AND (animal* OR livestock* OR swine* OR pig* OR poultry* OR chicken* OR cattle* OR dairy* OR beef*)]. A wildcard "*" was used to find plurals and word variants, and "multiple terms" used to find similar concept according to the website guidelines [24]. All retrieved records were saved for further review. Publications not reporting original research data, or written in languages other than English were further excluded. Publications containing AMU data in the abstract were selected and their full content was reviewed. Publications were broadly classified by the country where the research took place, and further categorized into whether they were carried out in a LMIC or a high-income country (HIC), based on the World Bank country classification for 2016 [25].

2.2. Data Extraction

From each selected publication, the following information was compiled as separate records (data points): (1) country of study; (2) year; (3) study unit (farm/veterinarian/veterinary prescriptions/sales data); (4) number of study units; (5) animal production type: level 1 (species),

cattle, poultry, swine, all species combined; level 2, beef cattle, dairy cattle, calves, heifers, broilers, layer chickens, turkeys, weaners, finishing pigs, adult pig/sows; (6) observation period (in months); (7) purpose of usage (non-specified/prophylactic/therapeutic/growth promotion); (8) route of administration (oral/water/feed/injectable/intra-mammary); and (9) source of data in the original publication.

The qualitative data included the reported 'prevalence of use' of antimicrobials/antimicrobial classes, or the relative distribution of antimicrobials sold. Quantitative data indicated the amounts used reported, in addition to the relevant expression units. All data were entered as single records ('data points') in Excel (Microsoft Office). Antimicrobials and antimicrobial classes listed were those included in the World Organisation for Animal Health (OIE) classification: veterinary critically important antimicrobial (VCIA) agents (10 classes); veterinary highly important antimicrobial (VHIA) agents (8 classes); and veterinary important antimicrobial (VIA) agents (8 classes) [26].

2.3. Data Analyses

We further analysed AMU data at farm level, and excluded information from studies based on veterinary prescriptions or pharmacies. AMU estimates from the same study, on the same animal species but on different years, different routes of administration, different production phases, or different types of use, were consolidated into a single data point. The usage rate (probability of use per month) (*UR*) was solved from the standard epidemiological formula:

$$P = 1 - e^{-UR \times t}$$

Therefore,

$$UR = -\frac{\log(1-P)}{t}$$

where P is the reported prevalence of usage (cumulative incidence) and t is the reported period of observation (months) [27].

The median (and 75% interquartile range) of the reported *UR* for each of the 10 most used classes of antimicrobials were calculated for cattle, poultry, and swine data.

For quantitative studies, the type of numerator, the population at risk, and the mathematical expressions used to quantify AMU were compiled. The data corresponding to different antimicrobials were added up by class (using the metrics reported). Metrics corresponding to animal-time (i.e., the product of the number of animals times the number of observed time units) were converted to 'doses per 1000 animal-days' for swine, cattle (dairy, beef), and poultry. The median (and 75% interquartile range) were given. For antimicrobials where the median across studies was 0, the arithmetic mean and the standard deviation was reported. All analyses were carried out using R statistical software (The R Foundation, Vienna, Austria).

3. Results

3.1. Publications

A total of 658 scientific publications were identified using the search terms listed above. Of those, 390 contained original research and 362 were written in English. AMU data (both quantitative and qualitative) was included in the abstract of 144 publications, and all of them were examined. Ninety-two articles contained AMU data within the body of the publication, but three contained extrapolation estimates, rather than survey data [20,28,29], and were thus further excluded, resulting in 89 publications to be reviewed (Figure 1).

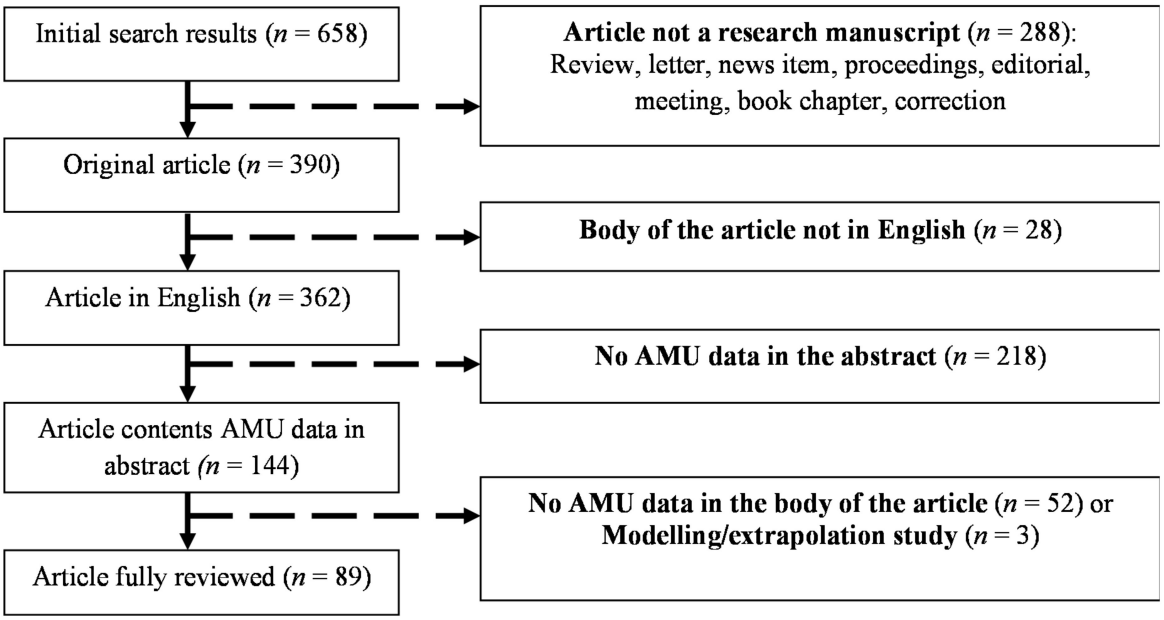

Figure 1. Selection and exclusion criteria for scientific publications on antimicrobial use (AMU) in animal production.

The 89 selected studies came from 29 countries (18 of which were classified as HICs and 11 as LMICs, according to the World Bank). Seventy-two (80.9%) studies came from HICs, and 17 (19.1%) from LMICs (8 from Asia, 7 from Africa, and 2 from the Americas). The countries with the highest volume of studies were Canada (11), Denmark (7), Belgium (6), and Germany (5). The studies were classified by publication year, country location, data source, and food animal species (Table 1).

Qualitative ('prevalence of use' of antimicrobials/antimicrobial classes, or the relative distribution of antimicrobials sold) and quantitative data (amounts of antimicrobial active ingredient) on AMU were reported in 46 and 60 studies, respectively. Seventeen (19.1%) studies reported both qualitative and quantitative data. Forty-eight percent of studies were published during the recent 2014–2018 period (70.6% of studies from LMICs). Over half (53%) of the studies were performed in Europe, followed by the Americas (23%), Asia (13%), Africa (8%), and Oceania (3%). About 38/47 (80.8%) of European studies reported quantitative AMU data, versus 9/18 (50%) studies from the Americas. A total of 66.3% studies were based on farm survey data, followed by 16.8% based on antimicrobial sales data. The most common animal species investigated were swine and cattle (43.8% studies), followed by poultry (24.7%). Ten percent of studies covered AMU in all species. Of the 17 publications from LMICs, only 7 (41%) reported quantitative data.

Table 1. Summary of 89 publications on antimicrobial use (AMU) stratified by year of study, country location, study design, and animal species, stratified by type of data (quantitative and/or qualitative) and type of country according to the World Bank income classification (2016). Individual studies are identified in the footnote (countries classified as low- to middle-income countries (LMICs) by the World Bank in 2016 are underlined). HICs—high-income countries.

Category	Sub-Category	Number of Studies (%)								
		HICs			LMICs			All Studies		
		Qualitative (*n* = 32)	Quantitative (*n* = 53)	All Types (*n* = 72)	Qualitative (*n* = 14)	Quantitative (*n* = 7)	All Types (*n* = 17)	Qualitative (*n* = 46)	Quantitative (*n* = 60)	All Types (*n* = 89)
Year of publication	2014–2018	9 (28)	26 (55)	31 (43)	10 (72)	6 (86)	12 (70)	19 (41)	35 (59)	43 (48)
	2009–2013	8 (25)	13 (24)	19 (26)	2 (14)	1 (14)	3 (18)	10 (22)	14 (23)	22 (25)
	2004–2008	12 (38)	8 (15)	17 (24)	2 (14)	0 (0)	2 (12)	14 (30)	8 (13)	19 (21)
	1998–2003	3 (9)	3 (6)	5 (7)	0 (0)	0 (0)	0 (0)	3 (7)	3 (5)	5 (6)
Country location *	Europe	13 (41)	38 (73)	47 (65)	0 (0)	0 (0)	0 (0)	13 (28)	39 (65)	47 (53)
	Americas	17 (53)	9 (17)	18 (25)	2 (14)	1(14)	2 (12)	19 (42)	10 (16)	20 (23)
	Asia	1 (3)	2 (4)	3 (4)	6 (42)	5 (72)	8 (47)	7 (15)	7 (12)	11 (12)
	Africa	0 (0)	0 (0)	0 (0)	6 (42)	1(14)	7 (41)	6 (13)	1 (2)	7 (8)
	Oceania	1 (3)	2 (4)	4 (6)	0 (0)	0 (0)	0 (0)	1 (2)	3 (5)	4 (4)
Study design	Farm survey	27 (84)	33 (62)	48 (67)	11 (79)	6 (86)	13 (76)	38 (83)	38 (60)	59 (66)
	Sales data	1 (3)	15 (28)	15 (28)	0 (0)	1 (14)	0 (0)	1 (2)	15 (24)	15 (16)
	Veterinarian survey	4 (13)	6 (11)	10 (19)	1 (7)	0 (0)	1 (6)	5 (11)	7 (11)	11 (12)
	Pharmacy survey	0 (0)	2 (4)	2 (4)	2 (14)	0 (0)	3 (18)	2 (4)	3 (5)	5 (6)
Animal species	Swine	11 (31)	25 (47)	36 (50)	3 (19)	1 (11)	4 (23)	14 (30)	26 (43)	39 (44)
	Cattle	20 (56)	23 (43)	36 (50)	3 (19)	2 (29)	3 (18)	23 (50)	27 (45)	39 (44)
	Poultry	5 (14)	11 (21)	13 (18)	7 (44)	5 (71)	9 (53)	12 (26)	16 (27)	22 (25)
	Combined data	0 (0)	5 (9)	5 (7)	3 (19)	1 (11)	4 (23)	3 (7)	6 (10)	9 (10)

* Europe, qualitative (13): Austria [30], Belgium [31], Germany [32], Norway [33], Italy [34–36], Spain [37,38], Finland [39], France [40]; UK [41,42], several EU countries [42]; Europe, quantitative (39): Denmark [43–49], Belgium [31,50–54], Germany [32,33,55–59] Austria [59–62], Switzerland [63,64], Netherlands [65–67], Sweden [68,69], France [40,70], Norway [33], Ireland [71,72], Italy [36], several EU countries [5,73], UK [74]; The Americas, qualitative (19): Canada [75–85], USA [86–91], Peru [92], Argentina [93]; The Americas, quantitative (9): Canada [75,80–85], USA [91,94], Argentina [93]. Asia, qualitative (7): Vietnam [95–97], Cambodia [98], Thailand [99], Japan [100], Iran [101]; Asia, quantitative (7): Vietnam [96,97,102], Thailand [103], Japan [6,104], Iran [101]. Africa, qualitative (6): Nigeria [105–108], Cameroon [109], Tanzania [110]; Africa, quantitative (1): South Africa [111]. Oceania, qualitative (1): Australia [112]; Oceania, quantitative (3): New Zealand [113–115].

3.2. Qualitative Data

Forty-six publications reported qualitative AMU data (Supplementary Material S1). These publications generated 50 data points on AMU by class, and 176 data points on use of specific antimicrobials. Data from 19 publications were not further analysed, because either the time frame was not provided, or the data presented reflected the distribution of different antimicrobials used or prescribed, not a prevalence of use. From the remaining 27 publications, 29 data points were compiled, corresponding to use of specific antimicrobials (11) or antimicrobial classes (18). Five data points corresponded to publications from LMICs (from poultry in Vietnam [96,97], Nigeria [105], Tanzania [110], and from cattle in Peru [92]. The usage rate (*UR*) (per month) for the most commonly reported antimicrobials and antimicrobial classes by type of animal production (poultry, swine, and cattle) is displayed in Figure 2.

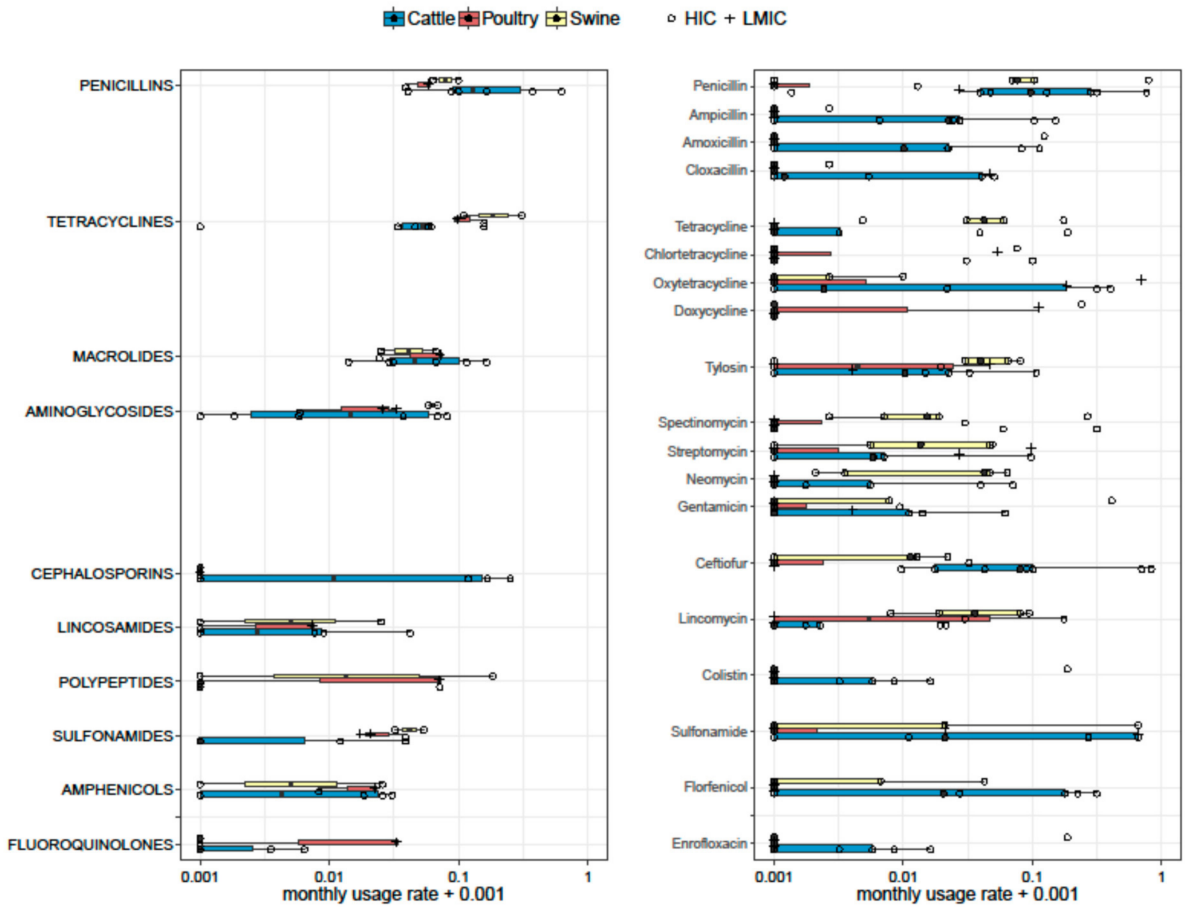

Figure 2. Boxplots representing monthly usage rage (*UR*) of antimicrobials (**Right**) and antimicrobial classes (**Left**). Six, three, and two estimates on antimicrobial classes were available for cattle, poultry, and swine, respectively. Nine, five, and four estimates on specific antimicrobials were available for cattle, swine, and poultry, respectively. The thickness of the boxes reflects the number of studies. HICs—high-income countries; LMICs—low- to middle-income countries.

Two, six, and three estimates on antimicrobial classes were available for swine, cattle, and poultry, respectively. In swine, tetracyclines had the highest *UR* (median 0.209; range 0.108–0.309), followed by polypeptides (0.091; range 0.000–0.183), penicillins (0.080; range 0.062–0.098), and aminoglycosides (0.062; range 0.057–0.067). In cattle, penicillins were the most frequently used antimicrobials in cattle with a median *UR* of 0.130 [inter quartile range (IQR) 0.090–0.320], followed by cephalosporins (0.058 [IQR 0–0.154]), and tetracyclines (0.051 [IQR 0.035–0.059]). The most used antimicrobial classes

in poultry were tetracyclines (median 0.095; range 0.095–0.156), followed by macrolides (median 0.071 [range 0.023–0.071]), polypeptides (median 0.069 [range 0.0–0.069]), and penicillins (median 0.057 [range 0.037–0.057]).

Five, nine, and four estimates on specific antimicrobials were available for swine, cattle, and poultry, respectively. Among studies reporting individual antimicrobials in pig farms, the highest *UR* corresponded to penicillin (median 0.075 [IQR 0.068–0.790]), tetracycline (0.041 [IQR 0.040–0.059]), neomycin (0.041 [IQR 0.003–0.046]), and tylosin (0.039, [IQR 0.029–0.063]). In cattle, penicillin was the most used antimicrobial (median 0.096 [IQR 0.039–0.291]), followed by ceftiofur (0.079 [IQR 0.013–0.40]), ampicillin (0.021 [IQR 0–0.060]), and sulphonamides (0.020 IQR [0–0.66]). In chicken farms, the most common antimicrobials used were doxycycline (0.056 [IQR 0–0.605]), followed by tiamulin (0.037 [IQR 0–0.90].

3.3. Quantitative Data

Accurate quantification of AMU in animal production requires the integration of two magnitudes, a 'numerator', and a 'population at risk' denominator (or 'target population'). The 'numerator' indicates the quantities of antimicrobial agent administered (farm surveys), prescribed (survey of veterinary practices), or sold (studies based on sales), in terms of the weight of antimicrobial, the number of animals treated, the number of treatment courses, or the number of animal daily doses. The 'population at risk' can be expressed as number of animals (expressed as animals produced, or a 'stationary' population census), bodyweight of animals (at slaughter or treatment), or 'animal-time' (the product of the number of animals times the number of observed time units) (Table 2).

Table 2. Classification of 60 publications reporting antimicrobial use (AMU) quantitative data by the type of metrics used and animal production types. Studies performed in LMICs are underlined. The number of publications reporting using those metrics is given in parentheses.

		Type of Animal Production (N)						All Studies (*N*)
	Population at Risk	**Dairy**	**Beef**	**Cattle (Unsp.)**	**Poultry**	**Swine**	**Total Use**	
Weight of antimicrobial	Animal-time	[63] (1)	[81] (1)	-	[97] (1)	[94] (1)	-	(4)
	No. animals produced	[69] (1)	-	-	[80,97] (2)	[47] (1)	-	(4)
	Weight of animal production	[104] (1)	[104] (1)	[101] (1)	[101–104] (4)	[44,102,104] (3)	-	(6)
	Weight of animal at treatment	[74,113] (2)	-	[64] (1)	[84] (1)	[60,61,64] (3)	[5,70,114] (3)	(10)
	Weight of animal time	[63] (1)	-	-	-	-	-	(1)
	No population at risk	[71,115] (2)	-	[5,43] (2)	[5] (1)	[5,43] (2)	[111] (1)	(5)
No. animals treated	Animal-time	-	-	-	-	[75] (1)	-	(1)
	No. animals produced	-	-	-	[33] (1)	-	-	(1)
No. treatment courses	Animal-time	[63] (1)	-	-	-	-	-	(1)
	No. animals produced	-	[40,41] (1)	-	-	-	-	(1)
No. daily doses	Animal-time	[30,31,53,63,65,69,72, 74,82,83,85,91,93] (13)	[51,67,81] (3)	[56] (1)	[51,52,67,84,96] (5)	[32,45–47,49–51,54–57, 60,62,66–68,116] (17)	-	(32)
	No population at risk	[58] (1)	-	-	-	[58] (1)	[59] (1)	(2)
No. studies		(18)	(5)	(5)	(13)	(27)	(5)	(60)

The most common quantitative metric was the 'animal daily dose' (ADD) [47,54,81], or a related expression such as the used daily dose (UDD) [54,57], the prescribed daily dose (PDD) [62], the animal daily dose x (ADD_x) [60,61], and the used course dose (UCD) [63]. In conjunction with an animal-time denominator, data on doses can be presented as a 'treatment incidence', which can be interpreted as the fraction of time over which animals are under treatment [49].

Thirty-two out of 60 studies reported AMU in animal daily doses related to animal-time, followed by studies reporting weight of antimicrobials related to the following: weight of animal at time of treatment (10), weight of animal production (6), animal-time (4), and number of animals produced (4). Five studies included quantitative AMU data, but the authors did not relate these to a population at risk. The formulae and calculations used in each publication are described in Supplementary Material S2. These 60 studies generated 939 data points related to total AMU use (528), AMU by class (310), and use of specific antimicrobials (108) (Supplementary Material S3). Only 7/60 (11.7%) studies were performed in LMICs.

Data from studies reporting animal daily doses were standardized as 'doses per 1000 animal-days' (equivalent to 'daily doses per 1000 animals'). Seventeen studies (all from European countries) reported AMU data in swine using these units. Two studies reported partial data (AMU in feeds only) [55,62]. Of the remaining 15 studies, eight reported 'overall' AMU on farms [45,47,50,51,54,61,67,116], whereas 7 reported AMU for specific age groups (sows, fattening pigs, suckling pigs, etc.) [32,46,49,56,57,66,68,117] (Figure 3). Across studies, pigs received a median of 40.2 doses per 1000 animals per day (or per 1000 animal-days) [IQR 8.5–120.4]. However, there were differences depending on whether the figures quantified overall (or average) farm AMU, or usage targeted to specific age groups within farms. Data from four studies reported a median of 134.2 [IQR 79.7–134.5] doses per 1000 pig-days for suckling piglets [57,58,70], 8.5 [range 7.9–30.4] to sows/adult pigs [47,57,70,117], and 29.6 [IQR 17.0–34.9] to fattening/finishing pigs [46,50,57,58,68,70]. In decreasing order, the following antimicrobials were given: penicillins (median 10.1 [IQR 2.7–39.7]), trimethoprim-sulphonamides (median 0.10; [IQR 0–31.2]); tetracyclines (median 5.6; [IQR 0–13.8]); macrolides (median 6.1 [IQR 0.16–16.7]); polymyxins (median 0 [IQR 0–7.1]); third generation cephalosporins (median 0.6 [IQR 0–10.6]), aminoglycosides (median 0, [IQR 0–0.2], mean 1.7; SD $\pm$ 3.5); and lincosamides (0 [IQR 0–0.5], mean 1.5; SD $\pm$ 4.0). Other antimicrobials were used less than 1 mean dose per 1000 pig-days. Antimicrobials in pigs were predominantly administered through the oral route, rather than through the parenteral route [50,54].

Thirteen studies reported dose-based data from dairy farms. All studies came from Europe, except one each from Argentina [93], the USA [91], and Canada [85]. One study reported AMU in heifers before calving [69], and another one reported AMU to treat mastitis [91] exclusively. One study reported separate data for calves, heifers, and dairy cows [63]. The remaining 10 studies reported overall farm AMU (Figure 3). The median number of doses reported in adult cattle was 10.0 doses per 1000 cow-days [IQR 5.5–13.6]. The most used antimicrobials were as follows (in decreasing order): penicillins (median 4.7 [IQR 1.8–5.8]); third generation cephalosporins (median 1.4 [IQR 0.1–2.1]); first generation cephalosporins (median 0.7 [IQR 0.1–0.9]); fourth generation cephalosporins (median 0.1 [IQR 0–1.9]); and aminoglycosides (median 0.6 [IQR 0–1.1]). Five publications reported AMU data as dose-based units in poultry, including three from Europe [51,52,67], one from Canada [84], and one from Vietnam [96]. One of the European studies only reported total use data [51], and data from the remaining four studies are shown in Figure 3. Except the study from Vietnam, which included small- and medium-scale chicken farms, other studies reported data from industrial broiler farms. The median AMU reported was 138 daily doses per 1000 chicken-days [IQR 91.1–438.3]. The Canadian study included in feed antimicrobial growth promoters (AGP) bacitracin and streptogramins, whereas the Vietnamese study did not. AGPs were banned in Europe at the time of the two other studies reported. The most commonly reported antimicrobials were penicillins (median 51.1 [IQR 40.1–52.9]), macrolides (median 33.0 [IQR 17.3–55.4]), trimethoprim-sulfonamides (median 25.0 [IQR 11.4–53.7]), tetracyclines (median 3.8 [IQR 0–49.1]), and fluoroquinolones (median 4.8 [IQR 0–26.9]). Only three

studies reported dose-based metrics in beef cattle, of which two reported AMU in veal production in the Netherlands and Belgium [51,67]. A study on beef farms from Canada reported a range of 3.3 to 10.7 per 1000 animal days depending on the type of farm; highest in cow-calf farms, and lowest in mixed feedlot and cow-calf farms. The antimicrobials most commonly given were tylosin (oral) and tetracyclines (injectable) [81].

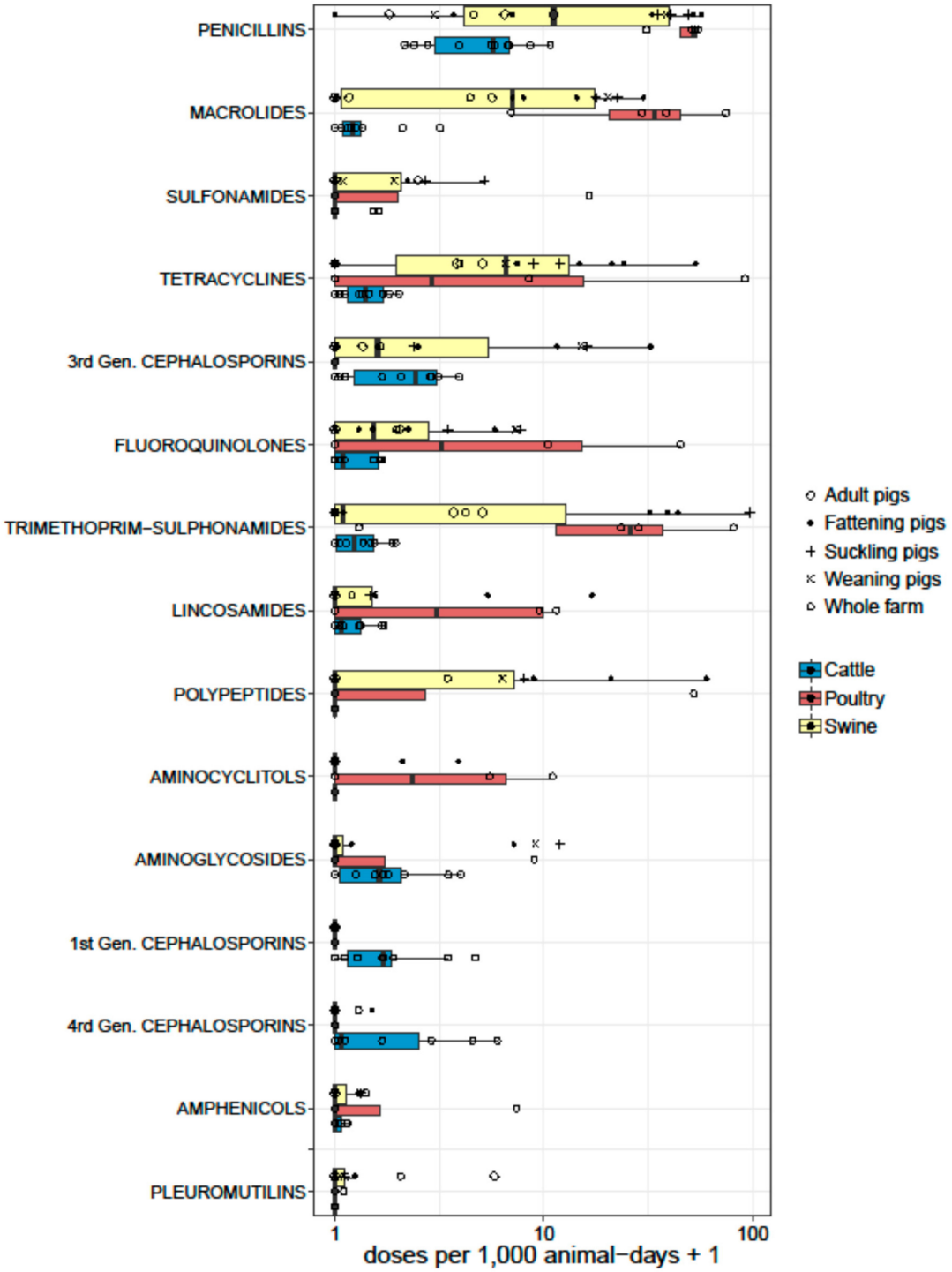

Figure 3. Boxplots representing the summary of AMU by antimicrobial classes from studies reporting quantitative data as doses (per 1000 animal-days) in swine (15), dairy (10), and poultry (4) farms. The thickness of the boxes reflects the number of studies.

A number of studies reported AMU related to weight of animal at treatment, standardized as 'population correction unit' (PCU) [50,60,61,64,70,74,84,113,114]. One PCU is equivalent to 1 kg of animal body mass at the time of treatment, which is set for each species (i.e., 1 kg for broilers, 65 kg for pigs, and 425 kg for cattle). A similar standardized measure is the LU ('livestock unit'). One LU was considered to be equivalent to 500 kg of animal biomass (i.e., one adult cow corresponds to ~1 LU, one fattening pig to ~0.15 LU, and one layer hen to ~0.004 LU) [59]. In a recent study, Danish researchers have proposed the use of an 'adjusted population correction unit' (APCU), which combines the PCU with the lifespan of the species treated, in order to reflect selection pressure of the antimicrobial over a kilogram of animal per unit time. Calculations using APCU demonstrated that PCU overestimated usage in short-living animal categories (i.e., poultry and, to a lesser extent, pigs), but underestimated AMU in long-living animals (i.e., cattle) [117].

4. Discussion

Here, we reviewed 89 studies on AMU in animal production published in English since 1998. In spite that LMICs are home to 84.2% of the global world population, only 17 (19%) publications came from such countries. This imbalance should be addressed, especially given that LMICs will account for the highest increase of AMU over coming years [20]. Interestingly, only one publication (from South Africa) was identified among all five BRICS 'emerging' economies (Brazil, Russia, India, China, and South Africa) [111]. It is highly likely, although could not be verified, that this somehow reflects a language bias, and some research has probably been published in languages other than English, or that falls outside the reach of the search engine. Most of the publications from LMICs were obtained from ad hoc farm surveys, as national AMU monitoring systems have not yet been established in most countries. A relatively small fraction of studies (7/17) included quantitative data.

Surveys based on a single farm visit may incur in recall biases, because often farmers do not keep records, especially in small-holder farms typical of many LMICs [118]. Although costly, longitudinal study designs where farmers are requested to keep records and/or antimicrobial product containers can potentially yield more accurate data than unannounced 'one-off' visits. However, there is also a risk that farmers may change their behavior or not provide accurate data, the latter being possible if farm visits are carried out by veterinary authorities that are perceived to negatively judge farmers' AMU practices.

Longitudinal study designs may allow insights into the seasonality of disease [57] and repeated behavior of consumption over time (especially when consecutive cycles of production are investigated) [65]. Such studies may also shed insights into treatment practices for different diseases or types of animal [46,83]. Finally, they may also allow to identify production types, farm sizes, and animal groups at higher risk of usage [45,116], as well as problems with over- and under-dosing [50,54,60,74]. Because longitudinal on-farm surveys are time-consuming and require considerable farmer commitment, they may be affected by a low response rate, limiting their representativeness [50]. In situations where there is a vast diversity of antimicrobial products, but the prevalence of use of each individual product is low, a small sample size may result in a 0 median [51,82], making results difficult to interpret. It would thus be preferable to report the mean and its associated standard deviation. The EU has recently issued recommendations on farm sampling strategies to investigate AMU at species level. These largely depend on the complexity and the size of the country. In the most complex situations (i.e. large countries with high heterogeneity of farming systems), a two-step cluster sampling procedure is recommended. It involves first, randomly selecting regions within the country (clusters), followed by stratification by farm type within each region, and systematic random sampling of farms with a selection probability proportional to their size. The EU also provides recommendations on required sample sizes [119]. In addition, the AACTING initiative aims to provide specific guidelines on monitor AMU at farm-level to monitor antimicrobial stewardship [120].

A number of publications (n = 10, of which 5 were from LMICs) reported prevalence of usage without providing a time frame, making interpretation difficult, because usage is dependent on the observation period. A further difficulty in interpreting prevalence of usage data is that in the studies reviewed, no information was provided as to whether antimicrobials were administered to whole flock/herds, or to individual animals. This is particularly relevant in large animal farming (i.e., pigs, ruminants), where individual treatment is common.

None of the studies from LMICs, except one from South Africa [111], included estimates on national sales. Sales data alone does not allow insights into species and production types at highest risk of use. However, if comprehensive, they can be useful to monitor general trends over time, provided that animal production figures remain stable. AMU data collated by national surveillance systems can be used to measure the impact of large-scale interventions, as performed in Norway and Switzerland after the EU compulsory withdrawal of AGPs [33,62], or changes in AMU over time due to the incursion of epidemics [44,49]. In recent years, the EU has implemented joint monitoring of AMU in humans and animals, although the data are mostly reported for all food animals combined [18]. Quantitative data on AMU in specific production types coupled with AMR data may potentially allow the elucidation of the relationship between AMU and AMR [119]. For countries with a considerable fraction of animal production aimed at the export market, it is imperative to include export data in the calculations [47].

As antimicrobials' active ingredients vary considerably in their potency, the use of dose-based metrics results in more fair comparison between antimicrobials. However, there is no universally accepted dose standards, as these vary by country, species, route of application, and indication [117]. Even if doses are standardized, estimating the number of doses from gross amounts of active ingredient is challenging because animals (especially poultry and pigs) may increase their body size over the production cycle for a factor of 50–100. For oral formulations (often given for flock/herd treatment), the feed and water intake needs to be estimated [80,96], and these data are rarely collected in small-holder farming systems typical of many LMICs. In situations when records are available, it is possible to contrast actual with theoretical use ($UDD_{animal}/ADD_{animal}$ or UDD_{kg}/ADD_{kg} ratios), and thus estimate the magnitude of over/under-dosing [54,61]. The change of technical specifications of doses may also lead to overall changes in AMU estimates, as shown in Denmark [47]. Comparing dose-based data (i.e., animal daily doses) across studies may present difficulties, because some report overall farm summaries, whereas others report AMU for specific subgroups (i.e., sows, piglets, calves).

In studies where weight and dose-based measures have been compared, some discrepancies have been found for some antimicrobials. For example, doses of tetracycline typically involve higher weights than polypeptides [57,81], fluoroquinolones, and cephalosporins [58]. Recently, the EU has standardized animal daily doses to encourage harmonized reporting across EU member states (termed defined daily dose for animals (DDDvet) [121].

For calculations at national level, animal-time denominator metrics should also take into account the length of empty periods on farms [67]. The definition of denominators based on weights at slaughter is challenging, especially because for long-living animals (i.e., dairy cows, sows, boars), only a small fraction of the standing population of these animals is slaughtered annually. This has been circumvented by using biomass data based on slaughter weight of animals for short living species (poultry, fattening pigs) and standing populations for long-living animals [104]. AMU has also been related to animal produce beyond meat (i.e., eggs and milk) [101]. Estimates of AMU related to food product could be used to define antimicrobial footprints to encourage responsible AMU in food animal production [103].

The European Union countries have agreed on the values assigned to PCU for animal species, which are used to standardize denominator data. However, animal production across the world is highly diverse, and this would require the definition of specific PCU values depending on the production systems. For example, the final slaughter weight of a traditional chicken in southern

Vietnam is 1.5–2.2 kg, whereas a typical broiler chicken may reach 2.6 kg. These values, as well as the variability in prescribing practices, are likely to affect the weight of animals at time of treatment.

Our review suggests a great variability in levels of AMU, between countries and species, as well as across age/production groups within species. Overall, AMU expressed as doses per unit of animal-time was highest in broiler production, followed by pig and dairy. An exception to this was a study from Belgium, where treatment incidence was higher in pig than in broiler production [51]. A study from Japan using estimates related to weight of animal production suggests that the amounts of antimicrobials used to produce 1 kg of pork far outweigh the amounts used to produce 1 kg of broilers or cattle [104]. This is likely to reflect the longer production cycle of pigs versus broilers (6 months vs. 1–1.5 months). Although adult cattle used generally fewer doses of antimicrobials per unit time, the use of critically important antimicrobials such as broad spectrum β-lactams and cephalosporins to treat mastitis infections is of great concern [30,31,83]. A considerable target of AMU in dairy cattle is the treatment of clinical mastitis and dry cow therapy [93]. We would like to highlight the lack of studies on AMU in poultry breeding flocks, laying flocks, and hatcheries worldwide. In some countries, it is common practice to dip or inject hatching eggs with antimicrobials to reduce the incidence of early infections [122].

This review confirmed a considerable deficit of studies on AMU from LMICs. Because of these data limitations, it cannot be concluded whether farms in LMICs are at higher or lower risk of AMU than their HIC counterparts. Also, it not clear to what extent animals in small-scale farms are raised using more or less antimicrobials than animals raised in larger (i.e., industrial) farms. There is conflicting evidence on this. One study from Vietnam showed higher levels of AMU in small- compared with medium-scale chicken farms [97]. Another study from the same country showed that pork, beef, and chicken meat samples purchased from wet markets were more commonly contaminated with antimicrobial residues than samples purchased from supermarkets. As supermarkets generally source their meat from industrial farms, this suggests higher levels of AMU in smaller farms [123]. However, another study on Thai pig farms reported the higher levels of antimicrobial usage in medium farms compared with small farms [99]. Although income limitations among farmers in LMICs may theoretically result in lower levels of AMU, in practice this may be offset by a higher incidence of infectious diseases, easier access to veterinary drugs, limited veterinary services, and generally looser legislative enforcement [124,125]. It is hoped that as more research/surveillance data on AMU in LMICs becomes available, this will become clearer.

5. Conclusions

We reviewed English-language scientific literature covering metrics and data pertaining to AMU in terrestrial animal production. Examination of these data indicates a considerable diversity of methodologies, as well as biases towards data from HICs and a concomitant data deficit from LMICs. Given the challenges posed by the variability of animal production systems, it would seem a priority to encourage the performance of on-farm surveys, and to recommend as a priority the collection of data as gross amounts (weight) of antimicrobial active ingredient by production system, and to further integrate these with production data collected at country level. The quantification of AMU using dose-based metrics should be carried out after the baseline data become available, but this requires standardization of dose definitions. In terms of treatment incidence, usage in poultry production is the highest, followed by AMU in swine and cattle production. We hope these data encourage the further investigation of AMU especially in LMICs with the aim of reducing the pressing threat of AMR worldwide.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-6382/7/3/75/s1.

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Conflicts of Interest: The authors declare no conflict of interests.

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Chapter 4

High resolution monitoring of antimicrobial consumption in Vietnamese small-scale chicken farms highlights discrepancies between study metrics

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High-Resolution Monitoring of Antimicrobial Consumption in Vietnamese Small-Scale Chicken Farms Highlights Discrepancies Between Study Metrics

*Nguyen Van Cuong[1], Doan Hoang Phu[1,2], Nguyen Thi Bich Van[1], Bao Dinh Truong[1,2], Bach Tuan Kiet[3], Bo Ve Hien[3], Ho Thi Viet Thu[4], Marc Choisy[1,5], Pawin Padungtod[6], Guy Thwaites[1,7] and Juan Carrique-Mas[1,7]**

[1] Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2] Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Ho Chi Minh City, Vietnam, [3] Sub-Department of Animal Health and Production, Cao Lãnh, Vietnam, [4] Department of Veterinary Medicine, University of Can Tho, Can Tho, Vietnam, [5] MIVEGEC, IRD, CNRS, University of Montpellier, Montpellier, France, [6] Food and Agriculture Organization of the United Nations, Hanoi, Vietnam, [7] Nuffield Department of Medicine, Centre for Tropical Medicine and Global Health, Oxford University, Oxford, United Kingdom

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Edited by:
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***Correspondence:**
Juan Carrique-Mas
jcarrique-mas@oucru.org

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Chicken is, among farmed species, the target of the highest levels of antimicrobial use (AMU). There are considerable knowledge gaps on how and when antimicrobials are used in commercial small-scale chicken farms. These shortcomings arise from cross-sectional study designs and poor record keeping practiced by many such farmers. Furthermore, there is a large diversity of AMU metrics, and it is not clear how these metrics relate to each other. We performed a longitudinal study on a cohort of small-scale chicken farms ($n = 102$) in the Mekong Delta (Vietnam), an area regarded as a hotspot of AMU, from October 2016 to May 2018. We collected data on all medicine products administered to 203 flocks with the following aims: (1) to describe types and quantities of antimicrobial active ingredients (AAIs) used; (2) to describe critical time points of AMU; and (3) to compare AMU using three quantitative metrics: (a) weight of AAIs related to bird weight at the time of treatment (mg/kg at treatment); (b) weight of AAIs related to weight of birds sold (mg/kg sold); and (c) "treatment incidence" (TI), or the number of daily doses per kilogram of live chicken [Vietnamese animal daily dose (ADDvetVN)] per 1,000 days. Antimicrobials contained in commercial feed, administered by injection ($n = N = 6$), or antimicrobials for human medicine ($n = N = 16$) were excluded. A total of 236 products were identified, containing 42 different AAIs. A total of 76.2% products contained AAIs of "critical importance" according to the World Health Organization (WHO). On average, chickens consumed 791.8 (SEM ±16.7) mg/kg at treatment, 323.4 (SEM ±11.3) mg/kg sold, and the TI was 382.6 (SEM ±5.5) per 1,000 days. AMU was more common early in the production cycle and was highly skewed, with the upper 25% quantile of flocks accounting for 60.7% of total AMU. The observed discrepancies between weight- and dose-based metrics were explained by differences in the strength of AAIs, mortality levels,

and the timing of administration. Results suggest that in small-scale chicken production, AMU reduction efforts should preferentially target the early (brooding) period, which is when birds are most likely to be exposed to antimicrobials, whilst restricting access to antimicrobials of critical importance for human medicine.

Keywords: antimicrobial use, chicken, small-scale farms, metrics, quantification, Vietnam

INTRODUCTION

Antimicrobial resistance (AMR) is a global threat to the health and wealth of nations (1). Antimicrobial usage (AMU) in animal production is regarded as a key driver of AMR in animal populations and a contributor to AMR in humans (2). AMU in animal production has been predicted to increase by 67% from 2010 to 2030 (3), while livestock production may increase by 74% between 1999 and 2030 (4). This increase is mostly driven by increased animal protein consumption in low- and middle-income countries (LMICs).

Chicken meat is the most consumed protein commodity in LMICs because of its comparative advantages. These include the relatively low capital investment and production costs, as well as the lack of religious objections to its consumption (5). In Vietnam, chicken meat currently ranks, after pork, the second most popular type of meat, and by 2020, it is forecast to surpass pork consumption (6).

In 2015, the World Health Organization (WHO) launched its Global Action Plan on AMR, with one of its key objectives being the development and enhancement of monitoring systems for AMU worldwide (7). However, measuring AMU in animal production in LMICs is often challenging due to the large numbers of small-scale farming units, high disease incidence, access of antimicrobials "over the counter," and generally loose regulatory framework (8). According to the Vietnamese official census (2018), of 245M chickens, only 26.1% corresponded to chickens raised in industrial systems (9), with the remainder corresponding to chickens raised in backyard and small-scale (semi-intensive) commercial farms.

AMU can be measured using a large diversity of metrics (10), and the choice of one metric over the other may lead to inconsistent results (11). Several studies have highlighted a very high level of AMU in Vietnamese chicken production, in terms of both frequency and quantities. A study in 210 poultry farms in northern Vietnam reported the use of 45 different antimicrobial active ingredients (AAIs) (12). A cross-sectional study in the Mekong Delta region indicated that, excluding feed, farmers used approximately 470 mg of AAIs to raise one chicken (13). In terms of treatment intensity, AMU in chicken flocks in a neighboring Mekong Delta province (Tien Giang) was 371 defined daily doses (DDD) per 1,000 chicken-days (14). Factors associated with such a high amount of AMU include ease of access to antimicrobials (i.e., density of veterinary drug shops) and the presence of disease and mortality in flocks, which has been described as very high (15).

However, most published studies in Vietnam (and in other LMICs) on AMU to date are based on cross-sectional study designs (i.e., a one-off visit) focused on the prevalent small-scale farm units. Since many farmers do not keep accurate records on AMU, they are likely to be prone to recall biases (16).

Using longitudinal active surveillance on a large cohort of small-scale commercial chicken flocks, we aimed (1) to describe the types of health-supporting products used, with a focus on antimicrobial active ingredients (AAIs); (2) to describe the critical time points for antimicrobial use (AMU) during the production cycle; and (3) to compare AMU using three common metrics of AMU in chicken production in the Mekong Delta of Vietnam. Detailed information about the types and timing of AMU, as well as its magnitude and the relationship between study metrics, is essential in order to improve the design of national/regional monitoring systems. Furthermore, this should help formulate more targeted campaigns aimed at promoting responsible use of antimicrobials among chicken farmers.

MATERIALS AND METHODS

Farms, Flocks, and Data Collection

The study was conducted from October 2016 to May 2018 during the baseline (observational) phase of a research project (17). Chicken farm owners of two districts (Cao Lanh and Thap Muoi) in the province of Dong Thap (Mekong Delta of Vietnam) were randomly selected from the official farm census held by the veterinary authorities (Sub-Department of Animal Health and Production of Dong Thap, SDAH-DT). These two study districts were chosen based on the availability of qualified veterinary staff to conduct the study. The two chosen districts have, on average, a human population of 331 and 354 chickens per square kilometer (2011); these figures are close to the average for the whole Mekong Delta region (410 humans and 478 chickens per square kilometer) (2011).

Farm owners registered in the census ($n = 207$) were convened and introduced to the project. Farmers intending to raise chickens in flocks of >100 chickens were invited to join the study prospectively as soon as they restocked their follow-on cycle. Project staff provided participating farmers with purposefully designed record books organized by week, where they were requested to record in detail the quantities of all health-supporting products used (including antimicrobial-containing products). Farmers were also asked to keep all packages (bottles, sachets, etc.) of any products purchased/used in their flock in a dedicated container. Study farms were visited four times during each flock production cycle to review the product containers (i.e., active ingredients, function, concentration, and instructions for use) and to verify the collected data. All data (commercial product names and quantities used) were entered into a database using a web-based application. The information

collected included number of chickens present in the flock each week and the number of chickens that died over the week. From these data, the flock cycle (cumulative) incidence of mortality was calculated for each production cycle by dividing the total number of birds that died during the period from restocking to sale by the total number of birds restocked for that cycle. A total of 203 flocks that completed at least one entire cycle (from 1-day-old chick until all chicken sold) raised in 102 farms were investigated. Of the 102 farms, 33 (32.3%) completed one cycle, 40 (39.2%) completed two cycles, 19 (18.6%) completed three cycles, 8 (7.8%) completed four cycles, and 2 (19.6%) completed five cycles. Recruited flocks ranged between 100 and 1,530 chickens at restocking. The median flock size at restocking was 300 [Interquartile range (IQR) 200–495]. The median duration of one production cycle was 18 [IQR 16–20] weeks, and the median cumulative mortality over the whole production cycle of flocks was 14.1% [IQR 6.8–29.2].

Description of Health-Supporting Medicinal Products

All health-supporting medicinal products were identified by their composition, and those products containing antimicrobials were singled out. They were described by type (human or veterinary medicine), composition (antimicrobial active ingredient only or mixed with other substances), number of active ingredients, administration route (drinking water, feed, injection), and formulation (powder, liquid). AAIs were classified based on the World Organisation for Animal Health (OIE) list of antimicrobial agents (18).

Timing of Antimicrobial Usage

The probability of a flock being medicated by age (production week) was calculated by dividing the total number of flocks where at least one antimicrobial-containing product was administered by the total number of flocks observed in the same week. In order to investigate potential seasonal variations in AMU, a Lexis diagram was created, with both the probabilities of AMU by production week and week calendar time plotted. A generalized logistic model was fitted with flock identity as the clustering variable and age and calendar week (sine and cos transformed) as covariates. The timing of AMU was investigated for the 20 most commonly used AAIs. The distribution of times of usage of each AAI from week 1 to week 21 (last week of AMU) was plotted.

Quantification of Antimicrobial Usage

The total live weight (body mass in kilograms) of chickens present in each flock at each week was calculated from the number of chickens present in the flock and their estimated weight. The latter was based on weekly weightings of 10 randomly-selected chickens from each of 11 representative flocks, from week 1 until week 22 of their production cycle (**Supplementary Data 1**). The amounts of AAI administered were calculated from farmers' records. The following two weight-based metrics were calculated: (1) weight of active ingredient related to the weight of bird at the time of treatment (mg/kg at treatment) and (2) weight of active antimicrobial active ingredient given over the whole production cycle related to weight of chickens sold (mg/kg sold). This was estimated from the number of chickens present in the flock and their weight at the time of sale. The instructions for mixing the products in water and/or feed (dilution factor) and the estimated daily water and feed consumption were used to estimate for each AAI the daily dose (in mg) associated with treating 1 kg of chicken (ADDvetVN). The weekly water consumption was estimated from the daily intake of a standard meat type pullet at an ambient temperature of 32°C (225 ml per kilogram of live chicken) (19); the weekly feed consumption was estimated from published data related to native Vietnamese layer pullets (i.e., 63.4 g daily per kilogram of live chicken) (20). The expressions used for the calculation of the above metrics are provided in **Supplementary Material S1**.

The number of ADDvetVN of each AAI administered on any given week to each flock (nADDvetVN) was inferred from the amounts of antimicrobial products consumed. The total nADDvetVN administered was divided by the duration of the cycle (in weeks) and multiplied by 1,000. This "treatment incidence" (TI) can be interpreted as the number of days (per 1,000 days) when one chicken is treated.

For antimicrobial products containing two or four AAIs, the number of doses (nADDvetVN) assigned to each AAI contained in the product was calculated as the total number of doses associated with the product divided by two or four, respectively. Products administered through the parenteral route (injection) and human medicines (tablets) were excluded, since the number of chickens receiving injection was not recorded, and guidelines for preparation of human medicines were not available. In addition, antimicrobials contained in purchased commercial feeds were not included in the analyses since they contained ambiguous formulations. Quantitative AMU metrics at the flock level were compared using Pearson's correlation coefficient (PCC). We calculated the mean and coefficient of variation of ADDvetVN values corresponding to AAIs present in Vietnamese antimicrobials and compared them with the DDDvet values defined for poultry by the European Medicines Agency (21).

RESULTS

Health-Supporting Products

A total of 619 different health-supporting products were identified among the 203 flocks investigated, of which 236 (38.1%) contained antimicrobials (**Table 1**). The most common non-antimicrobial health-supporting products ($n = 383$) consisted (in decreasing order) of vitamins/minerals (21.5%), digestive enzymes (8.1%), vaccines (3.7%), coccidiostats (3.6%), electrolytes (3.6%), anthelminthics (2.9%), and interferon/immunoglobulins (0.5%). Of the 112 "other" categories of product, most (∼80%) were anti-inflammatory/anti-pyretic products (i.e., paracetamol, prednisolone).

Of the 236 antimicrobial-containing products, 176 (74.5%) contained only AAIs (apart from excipient), whereas 25.5% contained AAIs mixed with other substances (i.e., vitamins, mineral, electrolytes, anti-inflammatory, and anti-pyretic

TABLE 1 | Summary of health-supporting products used by study flocks.

Type of product	No. of products (n = 619) (%)	Farms (n = 102) (%)	Flocks (n = 203) (%)	Weeks (n = 3,663) (%)
Antimicrobial-containing	236 (38.1)	100 (98.0)	192 (94.5)	933 (25.5)
Non-antimicrobial	383 (61.9)	102 (100)	202 (99.5)	2,128 (63.3)
Vitamins/minerals	133 (21.5)	99 (97.1)	189 (93.6)	1,428 (67.1)
Probiotics	50 (8.1)	86 (84.3)	157 (77.7)	942 (44.3)
Vaccines	23 (3.7)	102 (100)	203 (100)	784 (29.4)
Coccidiostats	22 (3.6)	76 (74.5)	137 (67.8)	304 (14.3)
Electrolytes	22 (3.6)	63 (61.8)	100 (49.5)	299 (14.1)
Anthelminthics	18 (2.9)	49 (48)	71 (35.1)	96 (4.5)
Interferon/immunoglobulins	3 (0.5)	88 (86.3)	144 (71.3)	293 (13.8)
Other (unclassified)	112 (18.1)	81 (79.4)	139 (68.8)	517 (24.3)

TABLE 2 | Description of antimicrobial-containing products administered to 203 chicken flocks.

Category	Sub-category	Products (n = 236) (%)	Farms (n = 102) (%)	Flocks (n = 203) (%)	Week (n = 3,663) (%)
Type of product	Animal medicine	220 (93.2)	100 (98.0)	191 (94.1)	697 (19.0)
	Human medicine	16 (6.8)	6 (5.9)	9 (4.4)	32 (0.9)
Composition	AAI only	176 (74.6)	92 (90.3)	169 (83.2)	629 (16.9)
	AAIs mixed with other substances	60 (25.4)	87 (85.3)	162 (79.8)	448 (12.2)
No. of AAIs per product	One	94 (39.9)	78 (76.5)	135 (66.5)	359 (9.8)
	Two	141 (59.7)	100 (98.0)	190 (93.6)	697 (19.0)
	Four	1 (0.4)	1 (1.0)	1 (0.5)	3 (0.1)
Administration route	Oral	227 (96.2)	100 (98)	192 (95.5)	928 (25.3)
	Oral—water	209 (88.9)	98 (96.1)	191 (94.1)	860 (23.7)
	Oral—feed	21 (8.9)	31 (29.4)	35 (17.2)	190 (5.2)
	Injection	6 (2.5)	13 (12.7)	14 (6.9)	19 (0.5)
Type of formulation	Powder	215 (91.1)	100 (98.0)	191 (94.1)	889 (24.3)
	Liquid	21 (8.9)	36 (35.3)	43 (21.2)	73 (1.9)

AAI, antimicrobial active ingredients.

substances). A total of 141 (59.7%) products contained two AAIs, and 1 (0.4%) contained four AAIs. Overwhelmingly, 227 products (96.2%) were intended for oral administration and 215 products (91.1%) were intended for powder-based formulations (**Table 2**). A total of 16 human medicine products were used by 4.4% of the study flocks. Antimicrobials were used in 25.5% observation weeks (n = 3,663).

Description of Antimicrobial Active Ingredients

A total of 42 different AAIs belonging to 13 classes were identified (**Table 3**). A total of 180 (76.2%) products contained antimicrobials of critical importance according to the WHO (22). Of those, 132 (55.9%) products contained AAIs of critical importance ("highest priority") and 91 (38.5%) products contained critically important ("high priority") antimicrobials. The most common AAI used were colistin (25.8% products, 83.7% flocks), followed by oxytetracycline (15.7%; 76.4%), tylosin (13.6%; 36.9%), doxycycline (11%; 30%), and amoxicillin (10.2%, 24.6%) (**Table 3**). Antimicrobials for human use consisted of tablets containing amoxicillin and tetracycline AAI (three products each); ampicillin, chloramphenicol, ciprofloxacin, and sulfaguanidine (two products each); and cefotaxime (one product). **Supplementary Material S2** includes the list of all AAIs contained in all antimicrobial products investigated.

Antimicrobial Use by Week

A Lexis diagram displaying the probability of AMU of flocks by production age and calendar time (weeks) is shown in **Figure 1**. The probability of AMU decreased with the age of the flock (from 0.76 in week 1, 0.41 in week 2, and 0.02 in week 21). From the Lexis graph, there was an indication of increased AMU during certain calendar periods (peaks in December 2016, June 2017, and February 2018). However, when both variables were fit into the same logistic model with the probability of AMU as an outcome, only the age of the flock (weeks) was significant (data not shown). A median of 5.0 [IQR 2.25–10.0] products and 6.0 [IQR 3.0–10.0] AAIs were used in each flock cycle.

TABLE 3 | AAIs administered to study flocks.

Antimicrobial class	AAI	Products ($n = 236$) (%)	Farms ($n = 102$) (%)	Flocks ($n = 203$) (%)	Weeks ($n = 3,663$) (%)
Aminoglycosides*	Neomycin	17 (7.2)	33 (32.4)	43 (21.2)	85 (3.1)
	Gentamicin	15 (6.4)	41 (40.2)	60 (29.6)	87 (3.2)
	Streptomycin	8 (3.4)	30 (29.4)	41 (20.2)	79 (2.9)
	Spectinomycin	7 (3)	10 (9.8)	12 (5.9)	18 (0.6)
	Apramycin	1 (0.4)	3 (2.9)	3 (1.5)	3 (0.1)
	Any aminoglycoside	50 (21.2)	69 (67.6)	115 (56.7)	259 (9.7)
Amphenicols	Florfenicol	13 (5.5)	24 (23.5)	27 (13.3)	40 (1.5)
	Thiamphenicol	3 (1.3)	20 (19.6)	27 (13.3)	36 (1.3)
	Chloramphenicol	2 (0.8)	2 (2.0)	5 (2.5)	15 (0.5)
	Any amphenicol	18 (7.6)	40 (39.2)	53 (26.1)	90 (3.4)
1st- and 2nd-gen. cephalosporins	Cefadroxil	1 (0.4)	1 (1.0)	1 (0.5)	2 (0.1)
	Cefotaxime	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Cefalexin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any 1st and 2nd gen. cephalosporin	2 (0.8)	2 (2.0)	2 (1.0)	4 (0.2)
Diaminopyrimidines	Trimethoprim	17 (7.2)	31 (30.4)	39 (19.2)	72 (2.7)
Lincosamides	Lincomycin	13 (5.5)	16 (15.7)	21 (10.3)	32 (1.2)
Macrolides**	Tylosin	32 (13.6)	48 (47.1)	75 (36.9)	160 (6.0)
	Tilmicosin	7 (3)	20 (19.6)	24 (11.8)	37 (1.3)
	Erythromycin	6 (2.5)	16 (15.7)	18 (8.9)	27 (1.0)
	Spiramycin	6 (2.5)	11 (10.8)	12 (5.9)	15 (0.5)
	Kitasamycin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Josamycin	1 (0.4)	2 (2.0)	2 (1.0)	4 (0.1)
	Any macrolide	51 (21.6)	57 (55.9)	91 (44.8)	227 (8.5)
Penicillins*	Amoxicillin	24 (10.2)	43 (42.2)	50 (24.6)	87 (3.2)
	Ampicillin	17 (7.2)	27 (26.5)	38 (18.7)	78 (2.9)
	Any penicillin	41 (17.4)	56 (54.9)	91 (44.8)	164 (6.2)
Pleuromutilins	Tiamulin	1 (0.4)	1 (1)	1 (0.5)	1 (0.0)
Polypeptides**	Colistin	61 (25.8)	94 (92.2)	170 (83.7)	413 (15.5)
	Enramycin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any polypeptide	61 (25.8)	94 (92.2)	170 (83.7)	414 (15.5)
Quinolones/fluoroquinolones**	Enrofloxacin	13 (5.5)	32 (31.4)	45 (22.2)	76 (2.8)
	Flumequine	9 (3.8)	12 (11.8)	16 (7.9)	27 (1.0)
	Norfloxacin	2 (0.8)	7 (6.9)	9 (4.4)	13 (0.4)
	Ciprofloxacin	2 (0.8)	2 (2.0)	3 (1.5)	5 (0.2)
	Marbofloxalin	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any quinolone	27 (11.4)	42 (41.2)	66 (33.5)	122 (4.6)
Sulfonamides	Sulphamethoxazole	7 (3.0)	26 (25.5)	34 (16.7)	68 (2.5)
	Sulfadimidine	6 (2.5)	8 (7.8)	9 (4.4)	11 (0.4)
	Sulfadimethoxine	6 (2.5)	14 (13.7)	16 (7.9)	21 (0.8)
	Sulfaguanidin	2 (0.8)	2 (2.0)	4 (2.0)	11 (0.4)
	Sulfadiazine	2 (0.8)	2 (2.0)	2 (1.0)	4 (0.1)
	Sulfamethoxypyridazine	1 (0.4)	2 (2.0)	2 (1.0)	4 (0.1)
	Sulfachloropyridazine	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Sulfamethazine	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Sulfathiazole	1 (0.4)	1 (1.0)	1 (0.5)	1 (0.0)
	Any sulfonamide	25 (10.6)	45 (44.1)	60 (29.6)	118 (4.4)
Tetracyclines	Oxytetracycline	37 (15.7)	87 (85.3)	155 (76.4)	332 (12.4)
	Doxycycline	26 (11.0)	42 (41.2)	61 (30.0)	129 (4.8)
	Tetracycline	6 (2.5)	7 (6.9)	10 (4.9)	28 (1.0)
	Any tetracycline	69 (29.2)	93 (91.2)	173 (85.2)	474 (17.8)
Unclassified	Methenamine	1 (0.4)	15 (14.7)	23 (11.3)	31 (1.1)

*Critically important antimicrobial classes according to the World Health Organization (WHO) are highlighted: *High priority, **Highest priority.*

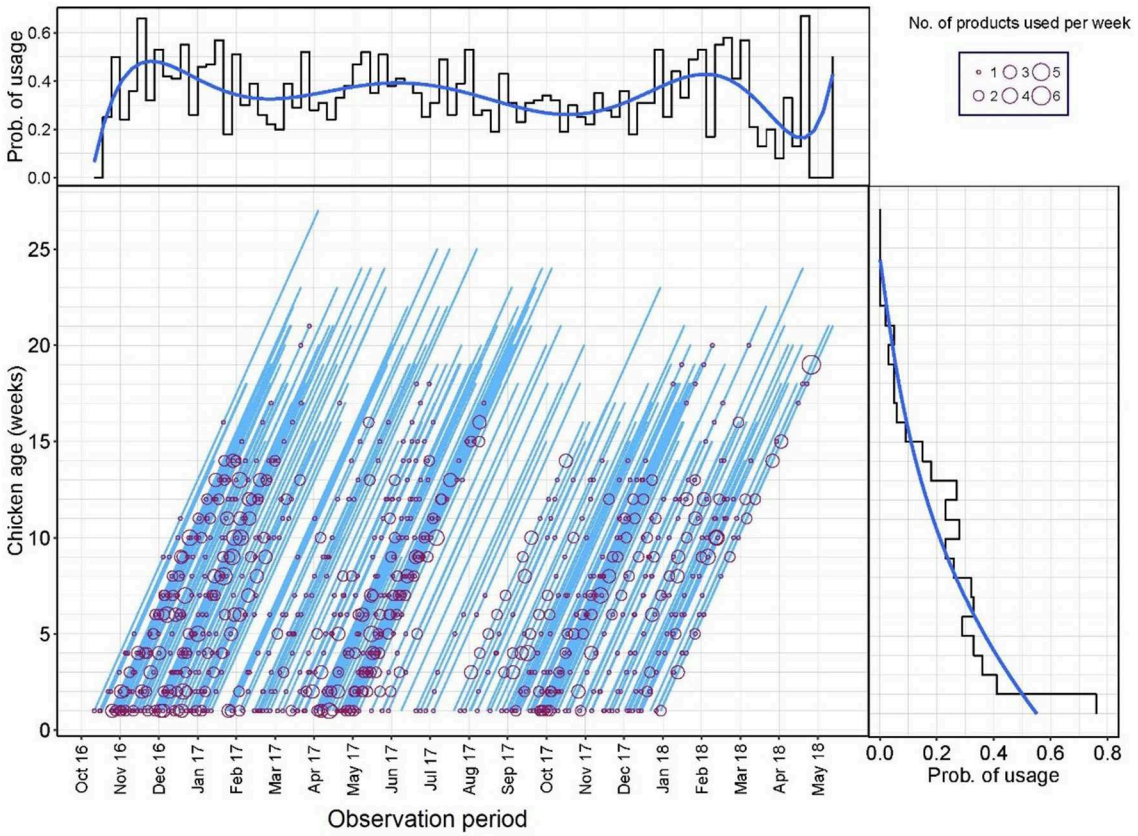

FIGURE 1 | Lexis diagram and probability of antimicrobial use (AMU) (Yes/No) by production week and calendar week during the study period.

Timing of Antimicrobial Use

In terms of timing of use, the AAIs used earlier in the production cycle were oxytetracycline [median timing of use, 2 weeks (IQR 1–5)], thiamphenicol [median 2.0 (IQR 1.0–6.0)], and colistin [median 3 (IQR 1.0–7.0)]. Tilmicosin [median 9 (IQR 6.0–12.0)], flumequine [median 9.0 (IQR 7.0–13.0)], and tetracycline [median 10.0 (IQR 6.0–12.0)] were the three AAIs that were administered latest to study flocks (**Figure 2**).

Quantification of Antimicrobial Use

Chicken flocks were administered a mean of 791.8 (±16.7) mg AAI per kilogram of chicken at treatment time [median 512 mg (IQR 264–1,094)] and 323.4 (±11.3) mg per kilogram of chicken sold [median 134 mg (IQR 62–279)]. The mean TI was 382.6 (±5.5) ADDs per 1,000 days [median 290 (IQR 125–583) per 1,000 days] (**Figure 3**). These calculations excluded AAIs contained in commercial feed, injectables, or human medicine antimicrobials. The data were quite skewed in all three metrics, with the mean being always greater than the median value. In terms of mg/kg at treatment, the upper 25% quantile of flocks accounted for 60.7% of total use. In addition, 23 (12.0%) flocks used more than 1,000 doses per 1,000 chicken days. For the "mg/kg sold" metric calculation, 9/203 (4.4%) flocks were excluded, since they experienced 100% mortality and therefore no live chickens were sold from such flocks.

Tetracyclines were the most used antimicrobial class reflected in both metrics: 285.1 mg/kg at treatment (SEM ±23.4) and a TI of 150.9 (±9.3) per 1,000 days. In terms of mg/kg at treatment, the highest magnitude of AMU corresponded to oxytetracycline 231.5 mg (29.2%), methenamine 105.8 mg (13.2%), and amoxicillin 48.7 mg (6.2%); in contrast, the highest TI corresponded to colistin 145.5, oxytetracycline 141.8, and enrofloxacin 16.1 (**Table 4**).

Correlation Between Antimicrobial Use Metrics

Figure 4 shows the three correlation plots between each pair of the three AMU metrics used. Correlation was highest between "mg/kg sold" and "mg/kg at treatment" (PCC = 0.457; $p < 0.001$) (moderate positive relationship). The metric "mg/kg at treatment" was weakly correlated with "treatment incidence" (PCC = 0.212; $p < 0.001$). There was no correlation between TI and mg/kg sold metric (PCC = 0.008; $p = 0.223$). The proportion of flocks with high mortality (≥14.1%) was significantly greater among flocks with higher than average AMU expressed with the mg/kg sold metric (0.64 vs. 0.34, $\chi^2 = 15.52$; $p < 0.001$). In the case of the other two metrics, there were no significant differences in mortality between high and low AMU users (both $p > 0.407$).

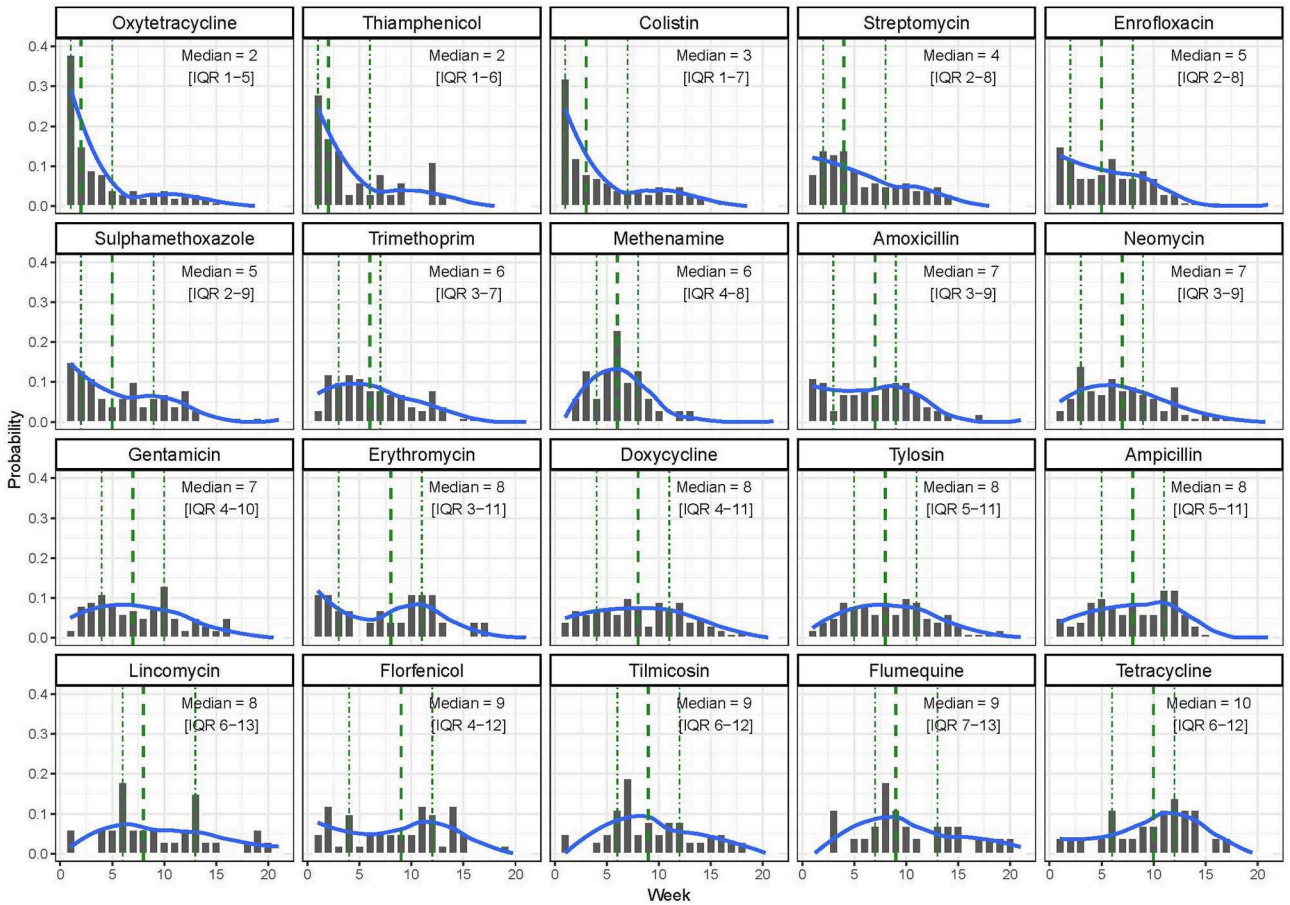

FIGURE 2 | Distribution of the timing of use of the 20 most common antimicrobial active ingredients (AAIs) by week among study flocks.

Vietnamese Animal Daily Dose for Chicken Production

The mean ADDvetVN corresponding to each of 37 AAIs was calculated from 223 different veterinary medicine products (**Supplementary Material S3**). ADDvetVN values ranged from 4.4 mg (sulfamethazine) to 320.6 mg (methenamine). However, most of the values were lower than 50 mg (35/38 AAI). A very high coefficient of variation (>100%) was also observed in several AAIs such as colistin, gentamicin, doxycycline, trimethoprim, tylosin, neomycin, spectinomycin, sulfadimidine, and florfenicol. There were 27 AAIs with data on DDDvet for poultry available in the European Union (EU). Of those, 14/27 antimicrobials from Vietnamese products had lower ADDs, while 13/27 had higher ADDs. Notably, the values of several DDDvet from the EU (i.e., spectinomycin, tylosin, ampicillin, and spiramycin) were four to five times higher than ADDvetVN.

Antimicrobial Use by Antimicrobial Active Ingredients

Figure 5 shows the correlation between TI and weight-based metrics (mg/kg at treatment and total weight of antimicrobials ignoring population treated) by AAI (**Supplementary Material S3**). The two metrics were moderately correlated (PPC > >0.480, $p < 0.001$ in both cases). However, the greater deviation from perfect correlation was observed for those AAIs with very low (i.e., colistin) or very high (i.e., methenamine) ADDvetVN values (5.2 and 320.6 mg/kg chicken, respectively). Comparing antimicrobials with similar TI, such as methenamine and spectinomycin (i.e., both ∼1 ADD per 1,000 chicken-days), given that the former has a much higher ADDvetVN value (320.6 mg/kg) than the latter (33 mg/kg), this results in quantitatively larger estimates for methenamine in terms of "total amounts (grams) of active ingredient" (**Figure 5**, right).

DISCUSSION

Our study deliberately focused on small-scale commercial farming systems. In doing so, we excluded both larger industrial (broiler) and backyard production systems. The small-scale commercial chicken sector represented here, alongside industrial broiler production, is increasingly important in Vietnam: from

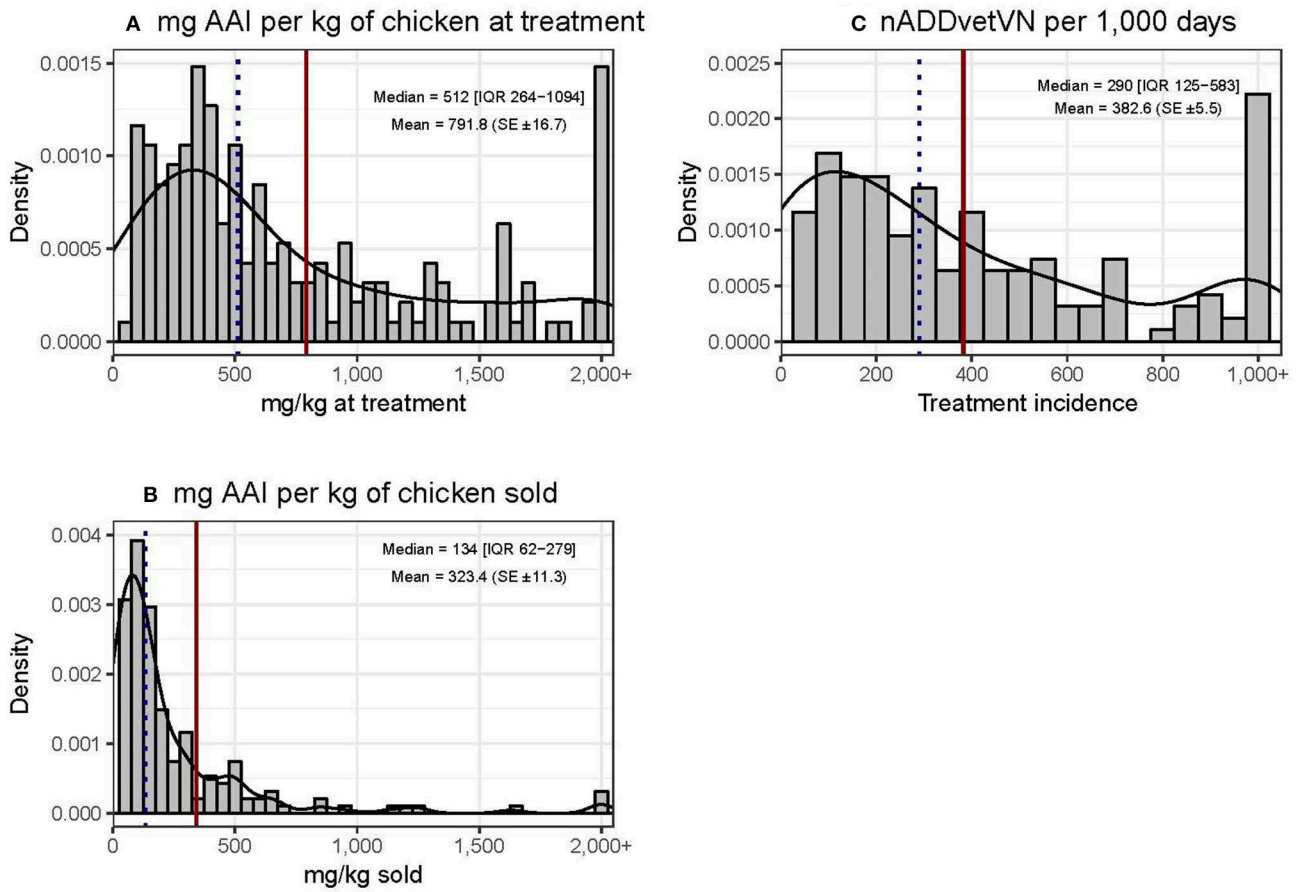

FIGURE 3 | Distribution of quantitative metrics of AMU in study flocks. Dotted black line: median. Solid red line: mean.

2011 to 2016 the number of registered units raising more than 100 chickens has experienced a 41.5% increase (23).

Using three different metrics, this study provided an accurate characterization of AMU in small-scale chicken flocks in the Mekong Delta of Vietnam, an area regarded as a hotspot of AMU. AMU levels were 791.8 (SEM ±16.7) mg of AAI per kilogram at treatment and 323.4 (SEM ±11.3) mg per kilogram sold. In terms of TI, chicken flocks were treated on average 382.6 days (SEM ±5.5) per 1,000 days. These results excluded antimicrobials included in purchased commercial feed formulations and a few antimicrobial products that were administered through the injectable route or human medicine antimicrobials products. In Vietnam, antimicrobials included in commercial feed have been quantified to be in the order of 77.4 mg per kilogram of live chicken raised in a previous study. In terms of TI, chickens in our study consumed three times more than global average levels (estimated in 138.0 doses per 1,000 chicken-days) (10).

It is particularly concerning that around three quarters (76.2%) of the products examined contained AAIs of "critical importance," and over half (55.9%) contained at least one AAI of critical importance (highest priority) according to the WHO (i.e., colistin, quinolones, and macrolides). The magnitude of colistin use is of particular concern, since this is one of the antimicrobials of last resort for hospital-acquired infections in humans (24). Colistin was found either alone or in combination with other antimicrobials such as oxytetracycline, ampicillin, neomycin, tylosin, enrofloxacin, etc. A possible reason for its popularity is its low cost, since it is an older-generation antimicrobial. Most (∼60%) antimicrobial-containing products were formulated with two AAIs. This scenario is different from European countries, where one active ingredient is allowed, except for a few drugs that are always formulated as combination (i.e., trimethoprim and sulphonamides) (21). In a small percentage of flocks (4%), we found that farmers had used chloramphenicol, an antimicrobial that has been banned for almost two decades in the country (25). In 2% of farms, ciprofloxacin (also banned for use in animal production) had also been used. We found a large number of farms that administered more doses than those technically necessary over the life of the flock. We believe that this is a reflection of errors in the preparation resulting in excessive concentration of the AAI during the early phases, since the costs of administering antimicrobials in small birds is relatively lower.

Results from this study highlight significant discrepancies between metrics. Relating AMU to chicken weight at treatment results in estimates of a magnitude two to three times higher than

TABLE 4 | Amounts of AAIs used through the oral route in study flocks.

Antimicrobial class	AAI	Mean AMU by flock (±SEM)		
		mg/kg at treatment	mg/kg sold	Treatment incidence
Aminoglycosides	Neomycin	38.0 (±16.4)	14.7 (±5.9)	4.4 (±1.1)
	Gentamicin	12.5 (±3.2)	6.3 (±3.5)	2.1 (±0.4)
	Streptomycin	22.5 (±10.5)	14.3 (±16)	6.0 (±1.3)
	Spectinomycin	2.2 (±3)	0.6 (±0.7)	1.0 (±1.0)
	Apramycin	0.5 (±1.1)	1.2 (±7.2)	<0.1 (±nc)
	Josamycin	0.9 (±3.2)	7.5 (±68)	<0.1 (±nc)
	Total aminoglycosides	75.7 (±5.9)	37.5 (±24.2)	13.5 (±2.7)
Amphenicols	Florfenicol	7.3 (±3.7)	9.4 (±12.1)	1.9 (±0.8)
	Thiamphenicol	26.2 (±12.5)	4.4 (±3.7)	3.1 (±0.6)
	Chloramphenicol	nc	nc	nc
	Total amphenicols	33.5 (±6.6)	13.8 (±1.2)	5.0 (±1.6)
1st and 2nd gen. cephalosporins	Cefadroxil	0.5 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Cefotaxime	nc	nc	nc
	Cefalexin	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Total	0.5 (±nc)	<0.1 (±nc)	<0.1 (±nc)
Diaminopyrimidines	Trimethoprim	25.7 (±nc)	11.7 (±nc)	4.3 (±nc)
Lincosamides	Lincomycin	3.2 (±nc)	2.3 (±nc)	1.4 (±nc)
Macrolides	Tylosin	34.8 (±8.5)	27.7 (±17.3)	6.5 (±1.2)
	Tilmicosin	25.9 (±19.2)	20.9 (±25.4)	7.8 (±4.6)
	Erythromycin	12.2 (±16.1)	5.7 (±12.3)	3.8 (±2.9)
	Spiramycin	1.5 (±1.4)	0.2 (±0.5)	1.1 (±0.5)
	Kitasamycin	<0.1 (±nc)	0.4 (±nc)	<0.1 (±nc)
	Josamycin	0.9 (±3.2)	7.5 (±68)	< 0.1 (±nc)
	Total	75.3 (±7.9)	62.0 (±10.4)	19.2 (±7.5)
Penicillins	Amoxicillin	48.7 (±24.7)	25.8 (±28.7)	14.4 (±3.4)
	Ampicillin	11.1 (±6.1)	5.5 (±4)	1.5 (±0.8)
	Total	59.8 (±13.2)	31.3 (±17.5)	15.9 (±7.5)
Pleuromutilins	Tiamulin	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
Polypeptides	Colistin	41.6 (±5.7)	8.8 (±1.6)	145.8 (±4.6)
	Enramycin	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Total	41.6 (±3.5)	8.8 (±0.9)	145.8 (±5.9)
Quinolones/Fluoroquinolones	Enrofloxacin	24.1 (±8.4)	7.4 (±4.6)	16.1 (±2.6)
	Flumequine	5.4 (±3.2)	3.4 (±2)	0.6 (±0.2)
	Norfloxacin	6.4 (±6.5)	2.4 (±3.5)	1.1 (±0.8)
	Ciprofloxacin	nc	nc	nc
	Marbofloxalin	nc	nc	nc
	Total	35.9 (±5.6)	13.2 (±4.8)	17.8 (±7.8)
Sulfonamides	Sulphamethoxazole	30.2 (±1.2)	11.7 (±15.1)	3.6 (±0.6)
	Sulfadimidine	4.1 (±4.8)	2.3 (±2.5)	0.1 (±nc)
	Sulfadimethoxine	13.5 (±27.7)	2.4 (±2)	1.9 (±1.4)
	Sulfaguanidin	nc	nc	nc
	Sulfadiazine	2.4 (±10)	0.7 (±4.8)	0.2 (±0.3)
	Sulfamethoxypyridazine	0.5 (±2)	0.3 (±0.8)	<0.1 (±nc)
	Sulfachloropyridazine	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Sulfamethazine	0.7 (±nc)	<0.1 (±nc)	1.0 (±nc)
	Sulfathiazole	<0.1 (±nc)	<0.1 (±nc)	<0.1 (±nc)
	Total	51.4 (±9.5)	17.4 (±5.1)	4.9 ±1.4)

(Continued)

TABLE 4 | Continued

Antimicrobial class	AAI	Mean AMU by flock (±SEM)		
		mg/kg at treatment	mg/kg sold	Treatment incidence
Tetracyclines	Oxytetracycline	231.5 (±21.0)	43.7 (±9.8)	141.8 (±4.6)
	Doxycycline	42.6 (±13.3)	14.0 (±3.4)	7.5 (±1.2)
	Tetracycline	7.4 (±46.8)	7.9 (±52.5)	1.6 (±4.0)
	Total	285.1 (±23.4)	65.6 (±27.9)	150.9 (±9.3)
Unclassified	Methenamine	105.8 (±nc)	58.0 (±nc)	1.1 (±nc)
Total		791.8 (±16.7)	323.4 (±11.3)	382.6 (±5.5)

nc, not calculated.

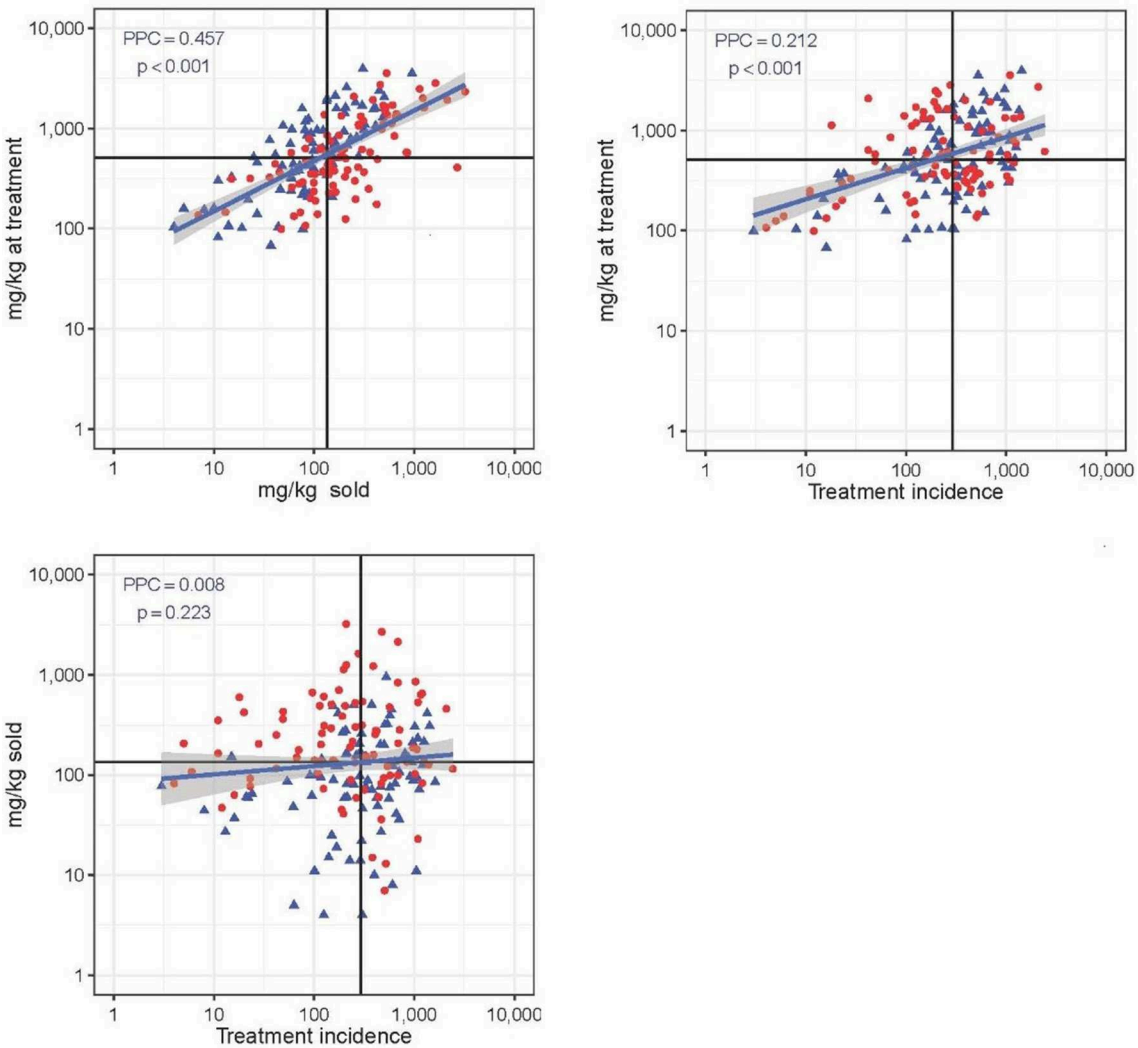

FIGURE 4 | Correlation between three quantitative AMU metrics ("mg/kg at treatment," "mg/kg sold," and "treatment incidence"). Solid black lines represent the median value of each metric. PCC is Pearsons's correlation coefficient. Red dot: flock with high (≥14.1%) mortality; blue dot: flock with low (<14.4%) mortality. Note the log 10-transformed scale for easier visualization.

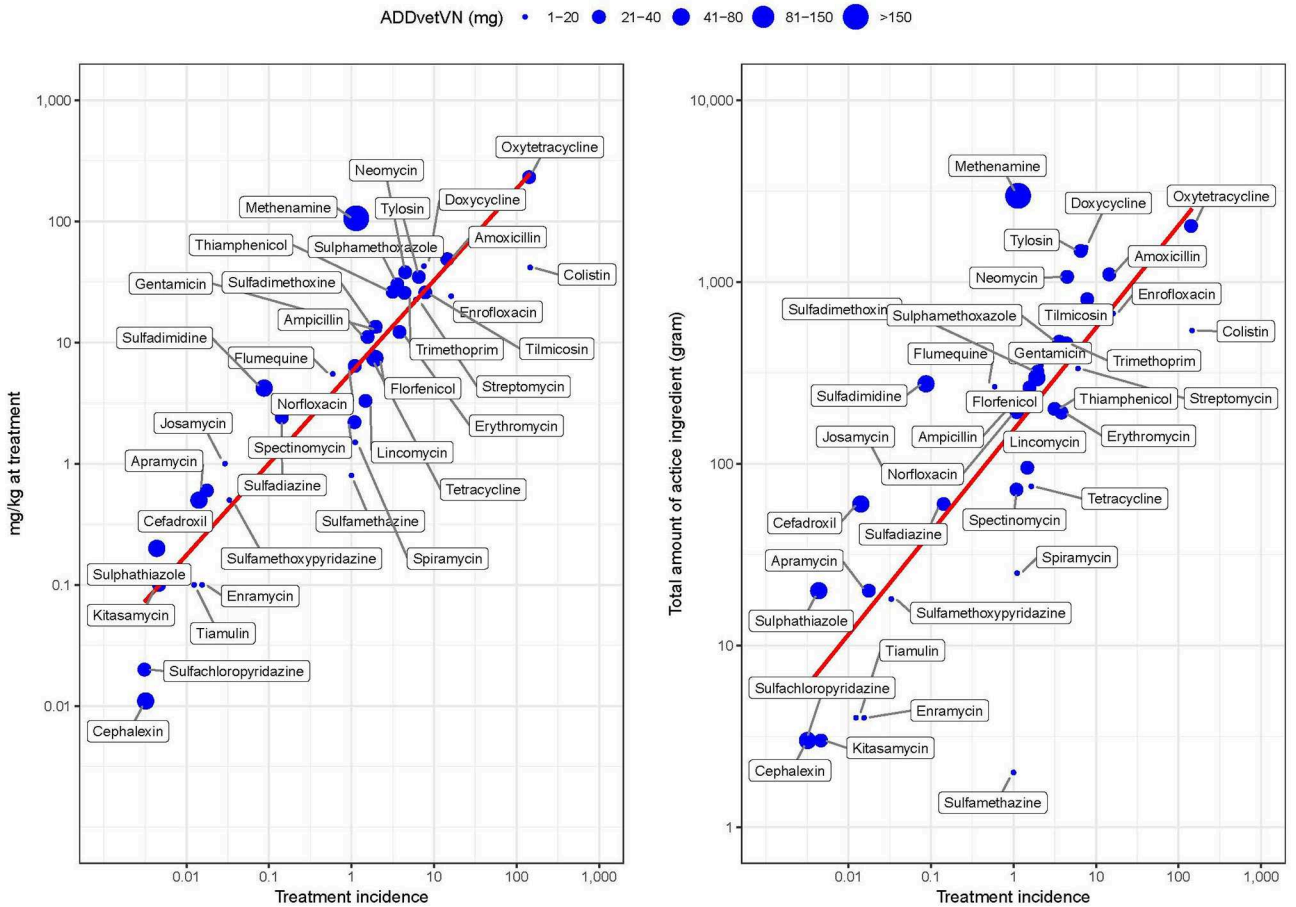

FIGURE 5 | Relationship between study metrics by AAI in study flocks. The size of the dot is proportional to the magnitude of the Vietnamese animal daily dose (ADDvetVN) for each AAI (in mg). Note the log 10-transformed scale for easier visualization.

relating AMU to chicken weight at the end of the production cycle. The "mg/kg at treatment" metric was largely influenced by the timing of AMU, with higher values resulting from administration of the product early in the production cycle (i.e., brooding), therefore resulting in larger estimates. The "mg/kg at treatment" use is expected to always be higher than "mg/kg sold," since the weight at the end of production is typically the highest. This latter metric was, however, largely affected by mortality, with flocks experiencing high mortality having considerably higher AMU estimates due to the smaller denominator in such flocks. If national estimates of AMU were to be calculated from production data, it is therefore essential to factor in the high levels of mortality that are typical of each production system. The "treatment incidence" metric is the most balanced overall metric, since it incorporates the variability associated with the variable strength of the AAIs administered. However, a challenge associated with the latter is the definition of a "daily dose," given that most antimicrobial products included guidelines for both prophylactic and therapeutic use, and information on the actual preparation procedures used by the farmer (dilution factor) was not collected. Indications for prophylactic use involve mixing the product with approximately half the strength of indication for therapeutic use. In addition, most products contain two AAIs, and each AAI amounted to half a theoretical daily dose in the overall calculation. The major discrepancies observed between weight-based and dose-based metrics can be explained because of differences in strength of different AAIs, timing of use, and variable mortality. In situations where AAIs characterized by large technical units are used, calculations using weight-based metrics will result in the overestimation of results using weight-based metrics over treatment incidence metrics.

We report differences in the timing of usage of different antimicrobials. Some antimicrobials, such as tetracycline and tilmicosin, have withdrawal times of over 1 week (26), and in several cases were administered late in the production cycle. This probably explains the high rate of detection of macrolide and tetracycline residues (10.3% each) in chicken meat samples purchased from the study area (27).

The study highlighted a huge diversity of AAIs used by small-scale chicken farmers. In Vietnam, about 10,000 products are currently licensed for veterinary use (28, 29), and ∼50% contain AAIs (author's observation). We established

the Vietnamese "animal daily dose" for antimicrobials used in chicken production (ADDvetVN). Athough our calculations of ADDvetVN were based on the indication displayed in the label for therapeutic purpose, most values were still lower than the DDDvet from the European Union, and for several AAIs (i.e., spiramycin, ampicillin) they were four to five times lower. In addition, many products included a recommendationa for prophylactic use, where the product is diluted by a factor of two, and the AAI is therefore administered at an even lower concentration. This is a concern, since such low doses may contribute to increased generation of AMR (30).

We are confident that farmers did provide an honest record of all antimicrobial products used and that the data collected in our study accurately represent AMU in these small-scale farming systems. This was possible since project staff were not perceived to judge farmers' practices negatively. However, obtaining longitudinal high-resolution data required several visits during the production cycle, and a considerable degree of both farmer and research staff commitment. Therefore, these types of studies may not be feasible at a large (i.e., national surveillance) scale, unless considerable resources are dedicated. We understand that the small-scale sector is the target of the largest quantities of AMU in Vietnam, and most of this use is for prophylactic purposes (15). This category of farmers should be the focus of policy makers to reduce excessive AMU in animal production. In Vietnam, most antimicrobials used in animal production are procured by farmers in licensed veterinary pharmacies. Because of this, we believe that setting up monitoring systems at these retail points, coupled with detailed animal production statistics (to be collected at local level), would represent a much more cost-effective surveillance system for AMU compared with conducting farm surveys.

Results highlight the need for training chicken farmers to improve their awareness on AMR while discouraging prophylactic use of antimicrobials, particularly during the brooding period. Such training should emphasize the need to improve day-old chick quality and farming practices (biosecurity, cleaning and disinfection, brooding management, and vaccination). Furthermore, in view of the high usage levels of AMU of critical importance (high priority), we recommend authorities to introduce phased restrictions, starting with those AAIs belonging to the highest priority group.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The ViParc project has been granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Minimal Risk) (Ref. 5121/16).

AUTHOR CONTRIBUTIONS

JC-M, PP, and NC conceived the study. NC, BK, BH, HT, and NV developed data collection methods and carried out field visits. NC, NV, MC, BT, and DP contributed to data analyses. NC, GT, MC, and JC-M contributed to manuscript writing-up and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00174/full#supplementary-material

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Chapter 5

Antimicrobial use through consumption of medicated feeds in chicken flocks in the Mekong Delta of Vietnam: a three-year study before a ban on antimicrobial growth promoters

Antimicrobial use through consumption of medicated feeds in chicken flocks in the Mekong Delta of Vietnam: a three-year study before a ban on antimicrobial growth promoters

Short title: Antimicrobials in commercial chicken feeds in the Mekong Delta of Vietnam

Nguyen Van Cuong [1], Bach Tuan Kiet [2], Bo Ve Hien [2], Bao Dinh Truong [1,2], Doan Hoang Phu [1,2], Guy Thwaites [1,4], Marc Choisy [1,4,5], Juan Carrique-Mas [1,4,*]

[1] Oxford University Clinical Research Unit, Vietnam

[2] Sub-Department of Animal Health and Production (SDAHP), Cao Lanh, Dong Thap, Vietnam

[3] Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, HCMC, Vietnam

[4] Centre for Tropical Medicine and Global health, Nuffield Department of Medicine, Oxford University, Oxford, United Kingdom

[5] MIVEGEC, IRD, CNRS, University of Montpellier, France

*Corresponding author:
Email: jcarrique-mas@oucru.org

Abstract

Antimicrobials are included in commercial animal feed rations in many low- and middle-income countries (LMICs). We measured antimicrobial use (AMU) in commercial feed products consumed by 338 small-scale chicken flocks in the Mekong Delta of Vietnam, before a gradual nationwide ban on prophylactic use of antimicrobials (including in commercial feeds) to be introduced over the coming five years. We reviewed the labels of commercial feeds and calculated amounts of antimicrobial active ingredients (AAIs) given to flocks. We framed these results in the context of overall AMU in chicken production, and highlighted those products that did not comply with Government regulations. Thirty-five of 99 (35.3%) different antimicrobial-containing feed products included at least one AAI. Eight different AAIs (avilamycin, bacitracin, chlortetracycline, colistin, enramycin, flavomycin, oxytetracycline, virginamycin) belonging to five classes were identified. Brooding feeds contained antimicrobials the most (51.2%), followed by grower (34.6%) and finisher feeds (12.5%). The average amount of AAIs given to flocks per kg of chicken at consumption time was 84.8 mg (SEM ±9.3mg). Quantitatively, chlortetracycline was consumed most (42.2mg/kg SEM ±0.34; 50.0% of total use), followed by enramycin (18.4 mg SEM ±0.03, 21.8%) and bacitracin (16.4mg SEM ±0.20, 19.4%). Antimicrobials in commercial feeds were more commonly given to flocks in the earlier part of the production cycle. A total of 10 (9.3%) products were not compliant with existing Vietnamese regulation (06/2016/TT-BNNPTNT) either because they included a non-authorised AAI (4), had AAIs over the permitted limits (4), or both (2). We estimated that consumption of antimicrobials in feed represented ~10% of total AMU (in feed and in water) in our study farms. A number of commercial feed formulations examined included colistin (polymyxin E), a critically important antimicrobial of highest priority for human medicine. These results illustrate the challenges for effective implementation and enforcement of restrictions of antimicrobials in commercial feeds in LMICs. Results from this study should help encourage discussion about policies on medicated feeds in LMICs.

Keywords: Antimicrobial Growth Promoters (AGPs), Commercial feeds, AGPs ban, Vietnam, small-scale, Chicken.

Introduction

Antimicrobials are used in veterinary medicine. The global annual consumption of antimicrobials intended for animal use has been estimated in the region of 63 thousand tonnes [1]. In European Union (EU) countries, all of which have well-developed antimicrobial consumption surveillance systems, antimicrobials intended for animal use quantitatively represent approximately 2/3 of total AMU [2]. It is believed that excessive use of antimicrobials in animal production is one factor contributing to the global rise in antimicrobial resistance (AMR), although its magnitude is unknown [3, 4]. The total amounts of antimicrobials intended for animal production are expected to increase in coming years due to intensification of livestock production, mostly in low- and middle-income countries [1]. Antimicrobials are used in veterinary medicine to treat and prevent animal disease. In addition, in many countries they are also added to feed rations in sub-therapeutic concentrations in order to increase animal growth and productivity (antimicrobial growth promoters, AGPs). Their mechanism of action is however, poorly understood [5].

Over the last years, the issue of AMR and excessive antimicrobial use (AMU) has attracted considerable attention worldwide. Many policy instruments on AMU/AMR have been recently developed by global organizations such as the World Health Organization (WHO) [6], the Food and Agriculture Organization of the United Nations (FAO) [7], and the World Organization for Animal Health (OIE) [8].

Antimicrobials in feed (including AGPs) have been the subject of much debate over recent years. Those opposing banning/restrictions of AGPs often express concerns based on potential losses in productivity, as well as the likelihood of emergence of certain diseases (i.e. necrotic enteritis in chickens) [9]. Positions in favour of their restriction often align themselves with the need to protect the efficacy of antimicrobials for human health. In the European Union (EU), mostly because of public health pressure, AGPs were banned in 2006 [10]. In recent years, and in line with FAO recommendations [7], some countries have started implementing bans or restrictions on AGPs in animal feeds, In the USA, voluntary phasing out of certain AGPs commenced in 2013 [11]. In the Asia-Pacific region, countries such as Korea (2011), Australia (2013) [12] have implemented bans of AGPs in animal feeds. Countries such as Thailand (2015) [13], China (2016) [14] and India (2019, Ministry of Health) have also recently adopted policies that restrict AGPs in commercial feeds.

Worldwide annual consumption of poultry meat (2013-2015) stood at 110,280 tonnes, second only to pork (117,005 tonnes). By 2025, chicken production is expected to surpass that of pork production [15].

In Vietnam, antimicrobials are often found in both commercial pig and poultry rations. A study estimated in-feed consumption of antimicrobials extrapolated from a retail survey of commercial feeds in 77mg of AGPs per kg of chicken produced [16]. A study of medium-sized chicken farms estimated that chickens consumed 57mg of AGPs per kg of animal produced [17]. However, that study was based on a small sample of 6 farms.

A 2002 Vietnamese government regulation on animal feeds (54/2002/QĐ-BNN) included a ban on 18 chemicals (including chloramphenicol, metronidazole and nitrofurans). Further (2014), legislation (28/2014/TT-BNNPTNT) expanded this list to bacitracin, carbadox and olaquindox. In May 2016 Vietnam issued Circular 06/2016/TT-BNNPTNT, listing those antimicrobial active ingredients (AAIs) authorized for inclusion in commercial feed types as AGPs, as well as the maximum levels allowed in each feed type. According to this regulation, the maximum number of different AAIs to be included in each feed was limited to two. In 2018, Vietnam introduced its Animal Husbandry Law (32/2018/QH14) generically banning the use of AGPs in commercial feeds. A further Decree (13/2020/ND-CP) included the timeframe for a ban on AMU for prophylactic purposes (including AGPs), with phased bans for different antimicrobials classes: WHO ‘highest’ and ‘high priority’ critically important AAIs to be banned from 2021, highly important AAIs from 2022, important AAIs from 2023 and all other antimicrobial classes from of 2026 [18].

This study aimed at investigating the types and quantities of AAIs in commercial feed in a large representative cohort of small-scale chicken flocks in the Mekong Delta region of Vietnam immediately before the implementation of the new Decree. This information complements existing data on antimicrobials administered in water administered by the farmer [19], and provides the full picture on antimicrobial consumption in small-scale commercial chicken flocks in the area. This knowledge should form the basis of informed decisions aiming at reducing AMU in animal production in Vietnam.

Materials and Methods

Farm selection

Farm owners in two districts (Cao Lanh, Thap Muoi) within Dong Thap (Mekong Delta, Vietnam) were randomly selected from the official (Sub-Department of Animal Health) farm census and were contacted by the veterinary authorities. Farmers about to start raising flocks of ≥100 chickens using native breeds that practiced all-in/all-out management were recruited, and flocks were followed up longitudinally. A total of 115 farms were recruited (59 in Cao Lanh; 66 in Thap Muoi). This study was performed in the context of a large field based trial aimed at reducing AMU in chicken production through the provision of veterinary advice [20]. Owners of selected farms were requested to record in detail the types of commercial feed used and to keep the sacs of all feed products used. A field study team visited farms four times over the production cycle to collect data on commercial feed products used by week. A total of 338 flocks raised in these farms were investigated. Of the 115 farms, 44 completed 1 cycle (38.3.4%), 25 (21.7%) 2 cycles, 8 (7.0%) 3 cycles, 11 (9.6%) 4 cycles, 12 (10.4%) 5 cycles, and 15 (13.0%) more than 5 cycles. The median flock size at restocking was 303 [IQR 200-500]. A total of 6,041 weeks of data were collected. The median duration of these native meat chicken production cycles was 19 [IQR 17-21] weeks. All farm visits were conducted from October 2016 to Oct 2019.

AAIs in commercial feed products

All commercial feed products containing an antimicrobial active ingredient (AAI) were singled out after reviewing their label. AAIs were described by: (1) target species (duck, chicken or pig); (2) indication by stage of production (brooder, grower or finisher); and (3) type of formulation (crumbs, mash or pellets). From each feed product, we described the AAIs contained and their concentration (expressed in mg/kg product). AAIs were classified based on the OIE list of antimicrobial agents [21] and any antimicrobials regarded as critically important by WHO [22] were highlighted. We excluded ionophore coccidiostats (aimed at controlling coccidial infections) since it is thought that these substances do not have a link with resistance against antimicrobials commonly used to treat human or animal bacterial disease. We identified those feed products containing antimicrobials at concentrations not permitted under Vietnamese legislation [23].

Data analyses

We calculated AMU consumption in feed by week by relating the amounts of AAI (mg) to the weight of birds at the time of consumption (standard weight of the flock) (kg) (mg/kg live chicken) for all weeks (*n*) over the flock's life duration (Expression 1).

$$\text{mg/kg chicken at time of consumption} = \sum_{k=1}^{n} \frac{AAI\ used\ (mg)\ in\ week\ k}{Standard\ weight\ of\ the\ flock\ (kg)\ at\ week\ k}$$

Weekly consumption of AAIs in feed was calculated by multiplying weekly feed consumption by the AAIs concentration indicated in that feed. The feed consumption was estimated from unpublished data related to native Vietnamese layer pullets, where 443g of feed were consumed by 1 kg of live chicken per week. The denominator (total weight of the flock at week *k*) was calculated from the number of chickens present in the flock multiplied by an estimated (standard) weight. The latter was based on weekly weight data from 10 randomly selected chickens from 11 representative flocks, collected from week 1 until week 22 of their production cycle [19].

The concentration (strength) of AAI in each feed product was obtained from its label. However, information for a number of feed products contained uncertain information in their labels, concerning the identity of the AAI and the amounts included. For feed products with AAI content ambiguously labeled (i.e. indicating inclusion of one of >1 listed AAIs), the amount of each AAIs was calculated by assigning each antimicrobial a probability corresponding being included (probability=1), and not being included (probability=0). For products indicating their AAIs concentration as a range, lowest and highest estimates were calculated for each antimicrobial. The amounts of each AAIs were summarized in each flock by AAI and by week. The total amounts of each AAI were aggregated to calculate total consumption by flock, including the estimation of a lower and upper limit from the above calculations.

Results

Description of commercial feed products

A total of 99 different commercial feed products were identified. Those products were intended for chicken (85 products, 85.9%), pig (12, 12.1%), and duck (2, 2.0%) feeding. Feed products intended for chickens were classified according to their indication (production stage): 38 for brooding, 22 for growing (i.e mid-production) and 25 for finishing. A total of 35 (35.3%) (all intended for chicken use) contained at least one antimicrobial. A total of 25 (65.8%), 9 (40.9%) and 5 (20%) commercial feeds intendend for brooding, growing and finishing, respectively, contained antimicrobials. Detailed information on all antimicrobial-containing feed products is available in S1 Table. All except one product (a brooder feed that contained both chlortetracycline and colistin) contained one AAI. A total of 12 (34%) products had an ambiguous label, indicating containing one of 2-4 listed AAIs. A total of 8 different AAIs belonging to 5 classes were listed in the 35 feed products. The most common AAIs listed were enramycin (18.8% feeds), followed by bacitracin (16.5% chicken feeds), chlortetracycline (15.3%), avilamycin (5.9%), flavomycin (4.6%), colistin (3.7%), virginamycin (2.4%), and oxytetracycline (1.2%) (Table 1). A total of 10 (9.3%) products were not compliant with Regulation 06/2016/TT-BNNPTNT, either because they included a non-authorised AAI (avilamycin, flavomycin, oxytetracycline) (n=4), AAI/s over the permitted limits (n=4), or for both reasons (n=2).

Table 1. Antimicrobial active ingredients (AAIs) and their concentrations in 85 chicken feed products given to flocks in Dong Thap (Mekong Delta, Vietnam).

AAIs	Class	Products (n=85) (%)	AAI mean concentration [range in mg/kg feed] (No. products)			**Permitted concentration [range in mg/kg feed]
			Brooder	Grower	Finisher	
Enramycin	Polypeptides	16 (18.8)	[7.7-10] (7)	[8.2-10.0] (5)	[11.6-11.6] (4)	[1-10]
Bacitracin	Polypeptides	14 (16.5)	[51.1-63.1] (8)	[125.0] (1)	[50-60] (5)	[4-50]
Chlortetracycline	Tetracyclines	13 (15.3)	[52.7-61.1] (9)	[40.0-50.0] (4)	-	[10-50]
Avilamycin	Orthosomycin	5 (5.9)	[12.5] (2) ††	[15.0] (1)	[10.0] (2) ††	NAA
Flavomycin	Other†	5 (5.9)	[6.0] (2) ††	[2.0] (1)	[10.0] (2) ††	NAA
Colistin*	Polipeptides	4 (4.7)	[70-136.6] (3)	-	[60.0-160.0] (1)	[2-20]
Virginamycin	Streptogramin A	2 (2.4)	[5.0-15.0] (1)	-	[5.0] (1) ††	[5-15]
Oxytetracycline	Tetracyclines	1 (1.2)	[50.0] (1)	-	-	NA

*Critically important antimicrobial class according to WHO. **AAIs permitted in chicken feeds from 1 to 28 day old birds (brooder and grower feeds) [24]. NAA = Not allowed antimicrobial. †Antibiotic complex obtained from *Streptomyces bambergiensis* and *Streptomyces ghanaensis*. ††All feed products with this AAI had the same strength.

AMU through commercial feed intake

All flocks were fed on commercial chicken feed. In addition, pig and duck feeds were given to 12.1% and 0.6% flocks, respectively. Each flock had been given a median of 2 [Inter-quartile range (IQR) 2-3] different commercial feed products. Flocks received a median of 1 [IQR 1-1] antimicrobial-containing products. Chickens were fed a mean of 84.8 (Standard Error of the mean (SEM) ±9.3mg/kg) [range 71.4-98.2] of AAI/kg over their production cycle. Chickens raised in Thap Muoi and Cao Lanh districts were given 87.7 (Standard Error of the mean, SEM ±14.8) mg/kg [range 76.1-99.3] and 81.7 (SEM ±11.0) mg/kg [range 66.3-97.1], respectively. Overall, the highest amounts of AMU corresponded to chlortetracycline (42.2mg, SEM ±0.34), followed by enramycin (18.4mg, SEM ±0.03) and bacitracin (16.4mg SEM ±0.20) (Table 2).

Table 2. AMU in commercial feed among 338 small-scale chicken flocks over 6,041 observation weeks.

AAIs	No. flocks (n=338) (%)	Prevalence of AMU by week (mean ± SEM) [lowest-highest]	Total AMU over the production cycle mg/kg chicken (mean ± SEM) [lowest-highest] (%)
Enramycin	152 (45.4)	0.319 (± 0.004) [0.306-0.333]	18.4 (± 0.032) [17.3-19.5] (21.8)
Chlortetracycline	73 (22.5)	0.134 (± 0.002) [0.134-0.135	42.2 (± 0.347) [40.6-43.9] (50.0)
Bacitracin	103 (30.5)	0.095 (± 0.014) [0.080-0.111]	16.4 (± 0.201) [10.5-22.3] (19.4)
Virginamycin	8 (2.9)	0.010 (± 0.032) [0.005-0.014]	0.5 (± 0.177) [0.1-0.8] (0.6)
Colistin*	7 (2.0)	0.005 (± 0.037) [0.003-0.008]	6.4 (± 4.217) [2.6-10.3] (7.6)
Avilamycin	8 (2.3)	0.005 (± NC) [0.0-0.010]	0.3 (± 0.088) [0.0-0.6] (0.4)
Flavomycin	8 (2.3)	0.005 (± NC) [0.0-0.010]	0.2 (± 0.115) [0.0-0.4] (0.2)
Oxytetracyline	4 (1.1)	0.0 (± NC) [0.0-0.001]	0.07 (± 0.731) [0.0-0.15] (0.1)
Total	297 (87.8)	0.575 (± 0.028) [0.529-0.624]	84.8 (± 9.390) [71.4-98.2] (100)

NC = Not calculated. *Critically-important antimicrobial class according to the World Health Organization.

Commercial feed rations were given to flocks over a total of 5,655 of 6,041 (93.6%) observation weeks. The probability of AMU in flocks decreased with the age of the flock (Fig 1a). On average, flocks were given AGPs in feed on 57.5% (SEM ±2.8%) weeks. Interestingly, a relatively high fraction of brooder products were used in later stages, while some finisher products were also used more in the growing period. Enramycin was used predominantly throughout the production cycle, while colistin was found only in later stages (Fig 1c).

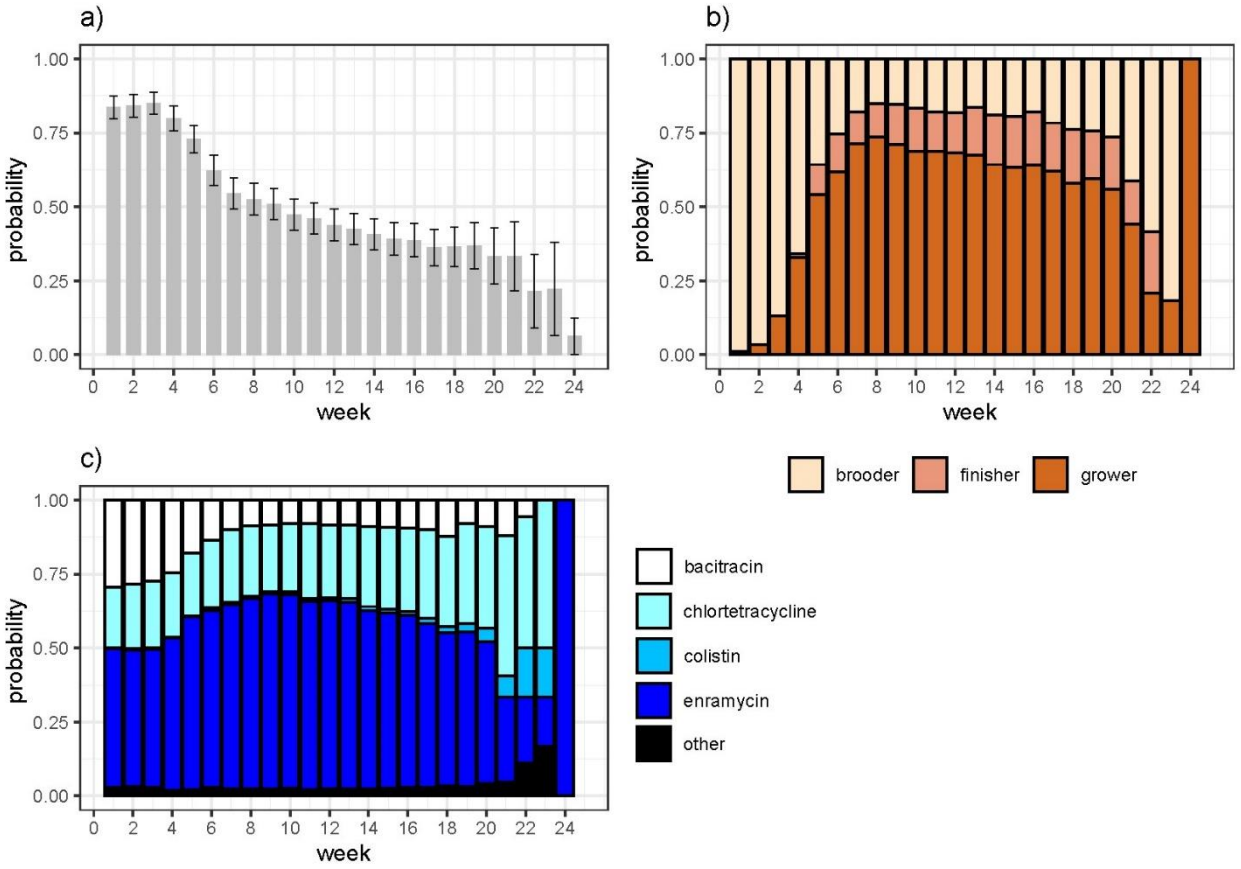

Fig 1: (a) Probability of consumption of AAIs in chicken feeds by week among study flocks; (b) Weekly distribution of types of feed (production stage) consumed by flocks; (c) Weekly distribution of AAIs consumed by flocks through commercial animal feeds.

Discussion

There are very few published data describing and quantifying consumption of AAIs in commercial feeds in poultry farming systems in LMICs [25]. Our findings complement existing data on antimicrobials administered (mainly through water) (~792 mg/kg) to Mekong Delta flocks [19].

Consumption of in-feed antimicrobials therefore represents ~10% of total chicken AMU (~85 mg/kg). This figure is consistent with previous estimates (77-95 mg/kg) [16, 17].

This study is based on data from a large cohort study aiming at reducing AMU in chicken production in the Mekong Delta of Vietnam [20]; therefore we believe that results are representative of commercially chicken farming systems, since selection of farms was random. Even though our data came from an intervention study, our advice to farmers was focused on reducing prophylactic and therapeutic administration of antimicrobials in water, and did not include advice on feed. Furthermore, we did not find any difference in in-feed antimicrobial consumption between flocks allocated to the intervention compared with the baseline phase (data not shown).

A major concern is the relatively high number of products that did not comply with Vietnamese regulations. Bacitracin, banned in feed rations in Vietnam since May 2016 [23], was the second most common AAI administered in feed. More worryingly, we found that 17% (6/35) antimicrobial-containing feeds included AAIs at concentrations higher than permitted by Vietnamese authorities. Notably, the strength of colistin was 3-5 times greater than permitted in all products examined, and non-authorised antimicrobials (avilamycin, flavomycin, oxytetracyline) were also found in some chicken feeds. This raises concerns regarding compliance of commercial feed mills with regulations, and casts doubts over the effective implementation of the phased bans [18]. An additional challenge is the ambiguous labelling of their AAI content in about a third of the rations investigated.

Recent studies have reported a high prevalence of colistin resistance encoded by *mcr-1* in chicken flocks in the area [17]. This antimicrobial, classified as highly critically important by WHO [26], was listed in 5% of feeds examined (brooder feeds) and we estimated that, on average, flocks consumed 5mg/kg of this antimicrobial (about 3% of total in-feed AMU). This is a modest amount compared with the reported magnitude of AMU through water administration (42 mg/kg). However, it is of concern that in our study farms these feeds were predominantly administerd towards the end of the production cycle, which may pose a risk of accumulation of antimicrobial residues in poultry meat [27]. It is of concern that only 16/35 (45.7%) AGP-containing feeds examined did not mention withdrawal times (data not shown). A recent survey showed that 8.4% of chicken meat samples in Vietnam contained antimicrobials residues, tetracyclines being the most common residue detected [28].

Quantitatively, in our study chlortetracycline, bacitracin and enramycin were the AAIs most consumed through commercial feeds. These results are not dissimilar to previous extrapolations from a retail survey in Vietnam [16]. Tetracyclines are also the most consumed antimicrobials consumed by flocks through water [19], and the antimicrobial class against which resistance among *Escherichia coli* and non-typhoidal *Salmonella* strains in the Mekong Delta is highest [17, 29, 30]. Bacitracin use has been shown to promote resistance among *Clostridium perfringens* isolates from chickens [31, 32]. With regards to enramycin, there is little information on its impact on AMR. A Japanese study that investigated *Enterococcus faecium* isolates from chicken flocks found no evidence of resistance against enramycin , although the study presented no enramycin use data [33].

Much of the debate on AMU in animals has often been framed in terms of bans on AGPs. Unfortunately, global data on total amounts of AGPs consumed or on the contribution of AGPs on total AMUare lacking. In Great Britain, in 2001 (5 years before the 2006 EU ban), AGPs represented 11.6% of 371 tonnes of antimicrobial active ingredients used in animal production [34]. We believe that AGPs represent a considerable fraction of total AMU globally, and probably these quantities have been decreasing over recent years, since more and more countries have phased out their use. A recent OIE survey reports that AGPs were used in 23% countries surveyed in 2018, compared with 51% countries in 2012 [35]. A review of the data of the impact of AGP from 1950 to 2010 on farm productivity indicate that productivity gains due to AGP in feeds decreased over the years [36], suggesting that any potential positive effects are of greater magnitude in low-biosecurity production systems. Indeed, recent studies in industrial (short cycle) broiler production systems showed that AGPs did not overall improve flock bodyweigth [37, 38], with one study resulting in a slight significant reduction over the whole cycle [38]. In the non-industrial production systems investigated here, antimicrobials in commercial feeds represented a relatively small fraction of total AMU. It is conceivable that even if AGPs may have lead to marginal productivity gains, these are likely to have been offset by the high mortality rates due to pathogen circulation common in the area [39].

Even though this study provides accurate quantification on consumption of AGPs in native chicken production in the country, more data are needed in order to accurately quantify levels of AGPs (and total AMU) in pig production, which by far for the most commonly consumed type of meat in Vietnam . Data from a survey in Vietnam suggest that in-feed antimicrobial consumpton in pig compared is of greater magnitude than in chicken production [16]. Similar to Vietnam, the use of

medicated feed in pig production in Thailand is common practice [40]. Because of a higher magnitude of use, the impact of reductions or bans on AGPs in the pig species is uncertain.

Conclusions

Compared with antimicrobials administered through water, antimicrobials in feed represent a relatively small fraction (10%) of total AMU in Vietnamese chicken production. However, it is of great concern that some feed formulations examined included colistin (polymyxin E), a critically important antimicrobial of highest priority for human medicine. Furthermore, a considerable number of feed formulations did not comply with Government regulations with regards to their AAI content, strength and/or withdrawal times, suggesting that effective enforcing and monitoring of such restrictions in Vietnam may be challenging; this situation is likely to be common to many other LMICs. It is likely that the types and quantities of antimicrobials in feeds vary by country and production system, therefore more data are needed to support targeted policy initiatives..

Declarations

Ethical approval and consent to participate

This study was granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Ref. 5121/16) and by the local authorities (People's Committed of Dong Thap province).Written informed consent was obtained from all participating farmers.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are provided as Supplementary Materials (S1 Table and S2 Data).

Competing interests

The authors declare no competing interests.

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Authors' contributions

NVC, BVH and JCM conceived and designed the study. BTK, DHP and NVC carried out data collection; NVC, MC and JCM performed data analyses; BTK, BDT contributed to data entry, NVC, BDT, DHP, JCM and GT contributed to writing up and editing. All authors read and approved the final manuscript.

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Chapter 6

Effects of prophylactic and therapeutic antimicrobial uses in small-scale chicken flocks

Effects of prophylactic and therapeutic antimicrobial uses in small-scale chicken flocks

Nguyen Van Cuong[1], Bach Tuan Kiet[3], Doan Hoang Phu[1,2], Nguyen Thi Bich Van[1], Vo Be Hien[3], Guy Thwaites[1,4], Juan Carrique-Mas[1,4], Marc Choisy[1,4,*]

[1] Oxford University Clinical Research Unit, Vietnam

[2] Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, HCMC, Vietnam

[3] Sub-Department of Animal Health and Production (SDAHP), Cao Lanh, Dong Thap, Vietnam

[4] Centre for Tropical Medicine and Global health, Nuffield Department of Medicine, University of Oxford, UK

***Correspondence:**

Marc Choisy

Phone: +84 3 38 99 78 96

Fax: +84 8 39 23 89 04

mchoisy@oucru.org

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Impact

- This study uses a large volume of observational data on disease and antimicrobial usage in small-scale chicken farms of the Mekong Delta region of Vietnam in order to quantify the prophylactic and therapeutic effects of antibiotics from 13 different classes on diarrhoea, respiratory infections, legs lesions and central nervous system infections.
- We show that prophylactic antimicrobial use never reduced the risk of diseases and that some classes actually increased the risk of some diseases (e.g. diarrhoea).
- In small-scale flock settings, the therapeutic use of antimicrobials leads to an increased in mortality in about 50% of the investigated antimicrobial/disease combinations.

Summary

Antimicrobials are extensively used both prophylactically and therapeutically in poultry production. Despite this, there are little data on the effect of antimicrobial use (AMU) on disease incidence rate and percent mortality. We investigated the relationships between AMU and disease and between AMU and mortality using data from a large (n=322 flocks) cohort of small-scale chicken flocks in the Mekong Delta, Vietnam, that were followed longitudinally from day-old to slaughter (5,566 observation weeks). We developed a parameterized algorithm to emulate a randomized control trial from observational data by categorizing the observation weeks into 'non-AMU', 'prophylactic AMU' and 'therapeutic AMU'. To evaluate the prophylactic AMU effect, we compared the frequencies of clinical signs in 'non-AMU' and 'prophylactic AMU' periods. To analyse therapeutic AMU, we compared weekly percent mortality between the weeks of disease episodes before and after AMU. Analyses were stratified by clinical signs (4) and antimicrobial classes (13). Prophylactic AMU never reduced the probability of disease, some antimicrobial classes such as lincosamides, amphenicols and penicillins increased the risk. The risk of diarrhoea consistently increased with prophylactic AMU. Therapeutic AMU often had an effect on mortality but the pattern was inconsistent across the combinations of antimicrobial classes and clinical signs with 14/29 decreasing and 11/29 increasing the percent weekly mortality. Lincosamides, methenamines and cephalosporins were the only three antimicrobial classes that always decreased the mortality when used therapeutically. Results were robust respective to the parameters values of the weeks categorization algorithm. This information should help support policy efforts and interventions aiming at reducing AMU in animal production.

Introduction

Antimicrobials play a critical role in the maintenance of animal health, animal welfare, and food-safety (FAO, 2016), and are used worldwide in food-producing animals for the prevention and treatment of infectious diseases. In addition, in some countries, antimicrobials are also added to commercial feed rations as growth promoters (Landers et al., 2012). Consumption of antimicrobials in animal production has been predicted to increase by two thirds from 2010 to 2030, of which one third is likely to include antimicrobial usage (AMU) for disease prevention and growth promotion purposes (or sub-therapeutic doses), especially in pig and poultry production (Van Boeckel et al., 2015).

In veterinary medicine, non-therapeutic administration of antimicrobials to individual animals is common in companion, bovine and equine medicine to prevent surgical site infections (Duclos et al., 2017; Dumas et al., 2016). In food animals, antimicrobials are often used to prevent bacterial infections (prophylactically) and also after potential exposure to a pathogen to reduce clinical signs and/or mortality (metaphylactically) (Pagel & Gautier, 2012; Rerat et al., 2012). Regardless of its purpose, in our study farms antimicrobials are typically administered to whole flocks via drinking water, making it difficult to distinguish therapeutic from metaphylactic use at flock level and both are generally indistinctly called therapeutic. Thus, in the rest of this article we define prophylactic or therapeutic use in relation to the use of antimicrobials before or after the onset of disease (i.e. clinical signs). Prophylactic AMU in poultry flocks often takes place during the brooding period and during other key events of the flocks' life such as vaccination and prior to transport. In a recent study of 203 small-scale commercial flocks (of 102 farms) in the Mekong Delta region of Vietnam, antimicrobials were extensively used and the highest frequency of AMU corresponded to the brooding period.

The practice of prophylactic medication of flocks/herds is likely to promote a shift in enteric bacterial populations from susceptible towards resistance. This is likely to have potential public health implications (Lugsomya et al., 2018).

There are limited data on the identity of pathogens circulating in the area. A study identified a range of global pathogens in diseased flocks in study area, the most common being, in descending order, *Avibacterium paragallinarum* (62.3% flocks), followed by *Mycoplasma gallisepticum* (26.2%), Infectious Bursal Disease (24.6%) and Infectious Bronchitis (21.3%). However, the diagnostic panel was limited to 9 pathogens and it is likely that many more pathogens are circulating in the area, and the pathogens are likely to change over time. In 47.5% of disease episodes more than one aetiological cause was found (BichVan et al., 2019). However, the exact reason for AMU (i.e. prophylactic versus therapeutic) in flocks remains unclear (Carrique-Mas et al., 2015; Cuong et al., 2019). Despite extensive use of antimicrobials in poultry production, there are little empirical data on the overall effects of prophylactic and therapeutic AMU on flock health. A recent study in Dutch layer chicks indicated that early mass prophylactic antibiotic treatment had a negative impact on adaptive immunity later in life (Simon et al., 2016).

Here we analysed observational data on AMU and disease (clinical signs) collected from a large cohort of small-scale chicken commercial flocks in the Mekong Delta of Vietnam (Cuong et al., 2019). We aimed to estimate: (i) the effect of prophylactic AMU on the subsequent probability of occurrence of a disease episode, and (ii) the impact of therapeutic AMU on subsequent mortality rate during a disease episode. In order to make causal inference from observational data we develop a parameterized algorithm that emulates a randomized control trial from these observational data, as recently proposed by Glass et al. (2013). We also explore the robustness of our results respective to the exact values of the parameters of our algorithm. The analyses were stratified by classes of

antimicrobial active ingredient (AAI) and type of clinical sign. These results provide a scientific basis that underpins policies aimed at reducing prophylactic AMU in farming systems.

Material and Methods

Data collection

Data on AMU, disease (clinical signs) and mortality from a random selection of commercial small-scale native chicken flocks raised for meat in Dong Thap province (Mekong Delta of Vietnam) were used. Farmers listed in the official census were initially contacted and invited to join the study. The data collection methods have been described elsewhere (Cuong et al., 2019). In brief, farmers were provided with a structured diary and were trained by project veterinarians to identify and record the most common clinical signs of disease, as well as to weekly record information on AMU and number of dead animals. The clinical signs recorded were: (i) respiratory distress (sneezing, coughing, nasal/ocular discharge, difficult breathing), (ii) diarrhoea (watery faeces), (iii) alterations of the central nervous system (CNS) (ataxia, circling, torticollis), and (iv) leg lesions (lameness, swollen joints/foot pads). Antimicrobial active ingredients (AAIs) were grouped by antimicrobial classes based on World Organization for Animal Health (OIE) criteria (OIE, 2015). A total of 5,566 weeks of data were collected from 322 flock cycles raised in 116 farms. The data were collected from October 2016 until May 2019. This is an observational study and thus did not require Institutional Review Board approval.

Analyses

The main challenge of the analysis consists in emulating a randomized controlled trial from our observational data. Below we explain in detail how this is performed.

The statistical unit in this study is a week of observation. The main challenge of the analyses is that antimicrobials were administered without mentioning the purpose of use (prophylactic or therapeutic). We thus had to use the information on the timing of presence of disease and AMU in order to categorize each week of the dataset into 3 categories: 'non-AMU' (used as control), 'prophylactic AMU' and 'therapeutic AMU'. Note that not all weeks could be assigned to one of these three categories as explained in the paragraph below that describes in detail the categorization algorithm.

For the prophylactic AMU analysis, we considered only weeks (i) without clinical signs reported during that week, as well as during the *y* preceding weeks, and (ii) without any antimicrobials being used during the *z* preceding weeks (filtering, step 0 on Figure 1). These selected weeks were then labelled as 'with AMU' or 'without AMU', depending on whether they had or not had AMU (exposure, step 1 on Figure 1) and, for each of these weeks, we computed the occurrence of clinical signs during the *x* subsequent weeks of observation (outcome, step 2 on Figure 1). The analyses were additionally adjusted for 3 covariables in order to control for potential confounders: (i) AMU during the first *a* weeks of the flock (brooding period), (ii) AMU during the *x* weeks of the observation period, and (iii) flock age (all in orange on Figure 1). Comparisons were performed by building a logistic generalized additive model with the probability of a disease episode as the dependent variable and in which the potential non-linear effect of age was modelled using a spline-based smoothing function, the optimal degree of which was obtained by cross-validation as implemented by the `mgcv` R package (Wood, 2017).

For the analysis of therapeutic AMU (i.e. therapeutic and metaphylactic combined), the statistical units were the weeks of an episode of disease, defined as a series of consecutive weeks with clinical signs recorded in a flock. Because clinical signs are likely to be under-reported, we allowed for the possibility of presence of weeks without any disease reporting in the middle of disease episodes. Figure 2 shows three examples of definition of disease episodes allowing gaps of 0, 1, and 2 consecutive weeks without any disease report. The weeks of disease episodes were then grouped into two arms (exposure): one with all the weeks (in blue on Figure 3) before the onset of AMU (if any, in red on Figure 3) in the disease episode, and the other one with all the weeks (in green on Figure 3) following onset of AMU (if any, in red on Figure 3). In case of absence of AMU during the disease episode, all the weeks were assigned to the first arm. In order to ensure that AMU can be considered as therapeutic, we excluded from the analysis all the weeks where other antimicrobials were used during the *p* weeks that preceded. The percent weekly mortality (proportion of chickens dying each week) were computed for the two arms and were compared using a logistic generalized additive model that included the spline-based smoothed age of the flock as a covariable as described above for the characterization of the prophylactic effect of AMU.

The two analyses included a number of tuning parameters. For the prophylactic AMU analysis, these were: *x*, the duration (in weeks) of the observation period; *y* and *z*, the numbers of weeks filtering for previous presence of clinical signs and AMU respectively; and *a*, the duration of the first few weeks of the flock during which we look for potential AMU. For the therapeutic analysis, we set a gap *g* (in weeks) to define disease episodes and *p*, the number of weeks filtering for previous AMU. Furthermore, in both analyses, disease is defined by the presence of at least one of a set of clinical signs, and AMU is defined by the use of at least one of a set of antimicrobials. In absence of information on what the values of these tuning parameters should be, we considered various combinations of them in order to assess the robustness of our results. For the prophylactic AMU analysis, we considered all the combinations (n = 27) of $x = 1, 2, 3$, $y = z = 1, 2, 3$, and $a =$

1, 2, 3. For the therapeutic analysis, we considered all the combinations (n = 9) of g = 0, 1, 2 and p = 1, 2, 3. We performed the analyses separately for each antimicrobial class (n = 13) and type of clinical sign (n = 4), as well for any AMU and any clinical signs.

Results

Data on AMU and clinical signs

Antimicrobials were administered to a total of 296/322 (91.9%) flocks and on 1,266/5,566 (22.7%) observation weeks. A total of 44 different AAIs corresponding to 13 antimicrobial classes were used, with tetracyclines, polypeptides, aminoglycosides, macrolides and penicillins being the most commonly used classes (both by flock and by week, table 1). In addition, clinical signs were reported on 530/5,566 (9.5%) weeks, with diarrhoea on 305 (5.5%) weeks, respiratory on 213 (3.8%) weeks, leg lesions on 71 (1.3%) weeks and CNS on 51 (0.9%) weeks.

Data for prophylactic and therapeutic AMU analysis

Depending on the values of the tuning parameters, 353-686 (27.9%-54.2%) of the 1,266 AMU weeks were classified as prophylactic AMU. The highest frequency of prophylactic AMU corresponded to tetracyclines, polypeptides, aminoglycosides and macrolides classes. A range of 1564-3251 (36.4%-75.6%) of all the 5,566 weeks was classified as non-AMU. A range of 144-310 disease episodes was identified. The highest frequencies of first week therapeutic AMU corresponded to tetracyclines, polypeptides, aminoglycosides and penicillins class. Ranges of 21-164 and 1-153 weeks was classified as weeks ‘before’ and ‘after’ therapeutic AMU respectively. The details of the data used for each class of antimicrobial is presented in Table 1.

Impact of prophylactic AMU on disease occurrence

Figure 4 shows the odds ratio (OR) of the effects of prophylactic AMU per antimicrobial class and clinical sign, and for all the combinations of the tuning parameters. None of prophylactic AMU ever protects (i.e. OR significantly below 1) from any of the clinical signs. On the contrary, in 10 of the 52 antimicrobial class x clinical sign combinations, prophylactic AMU actually increases the probability of occurrence of disease. Only the CNS was never affected. The risk of diarrhoea increased with the prophylactic use of lincosamides, methenamines and penicillines. The risk of respiratory infections increased with the prophylactic use of lincosamides and amphenicols. The significances of these effects are higher for short observation periods and longer initial period of flocks. The duration of the filtering period has little effect of the significance of these results.

Impact of therapeutic AMU on mortality

Figure 5 shows the odds ratio of the effects of therapeutic AMU on percent weekly mortality, stratified by antimicrobial class and clinical sign, and for all the combinations of the tuning parameters. Therapeutic AMU almost always has an effect on the mortality rate. However, this effect varies both between and within antimicrobial classes and clinical signs combinations. Out of the 31 combinations for which we have data, only 2 do not show any significant results. Among the 29 other ones, 11 showed robust increase in mortality rate (respective to the exact values of the tuning parameters), 14 showed robust decrease in mortality rate, and 4 showed inconsistent results depending of the values of the tuning parameters. The effects of the tuning parameters on the significance of the results were not consistent from combination to combination of antimicrobial classes and clinical signs. Lincosamides and methenamines always decrease the mortality and this is fairly robust respective to the exact values of the tuning parameters. AMU in response to leg lesions always increases mortality.

Discussion

Based on disease reporting data collected longitudinally from chicken flocks, our study suggests that prophylactic AMU does not protect against disease. Instead, we found that prophylactic AMU did increase the risk of disease in a number of situations. Specifically, we found that some of the antimicrobial classes administered prophylactically resulted in increased risk of subsequent diarrhoea (lincosamides, penicillins, methenamines, and tetracyclines classes) and respiratory infections (lincosamides, penicillins, amphenicols and macrolides). The association between AMU and diarrhoea has a biological basis, since microbial communities of the gastro-intestinal tract of chickens play an important role in nutrient digestion, pathogen inhibition and interact with the gut-associated immune system (Borda-Molina et al., 2018). These results are also consistent with previous studies: oral administration of clindamycin (lincosamide class) in humans results in considerable alterations of the intestinal microbiota even long after discontinuation of the antimicrobial course (Jakobsson et al., 2010). A study on pigeons receiving this drug resulted in an increased risk of secondary yeast infection, resulting in diarrhoea and sour crop (Lenarduzzi et al., 2011). Similarly, the therapeutic use of methenamines, tetracyclines and broad-spectrum penicillins in humans have been shown to have enteric side effects (Chwa et al., 2019; Rafii et al., 2008).

Our analyses also show that the significance of the effect of prophylactic AMU on clinical signs tends to decrease as the duration of the observation period increases, suggesting that the effect of AMU may be of relative short term, typically 2 weeks. It is believed that antibiotic treatment may trigger dysbiosis, which may impact host systemic energy metabolism and cause phenotypic and health modifications (Le Roy et al., 2019). Furthermore, a study indicated that bacterial phylotypes shifted after 14 days of antibiotic treatment in pigs (Looft et al., 2012), and 7 days in humans (Jakobsson et al., 2010).

The significance of the effect of prophylactic AMU on clinical signs increased with the duration of the brooding period we considered. AMU during the first weeks of life has been reported to decrease the diversity of intestinal microbiota, which may have health consequences later in life (Kers et al., 2018). It is not clear whether antimicrobials reduce the immune response of chicken, although a study in broiler indicates that hematological values fell after the administration of antibiotics to young chicks (1-5 day old) (Al-Saad & A.A. Yones, 2014).

Contrary to the effect of prophylactic AMU on disease occurrence, the effect of therapeutic AMU on mortality was almost always significant. However, the general picture was less clear-cut than for prophylactic effects as it varied greatly both within and between combinations of antimicrobial classes and clinical signs, as well as depending on the values of the tuning parameters. Therapeutic AMU always increases mortality for leg lesions. For the 3 other clinical signs, it depends on the antimicrobial class. Lincosamides and methenamines always decrease the mortality. The effects of the other antimicrobial classes depend on the clinical signs under consideration. Interestingly, lincosamides and methenamines are also two classes that confer the highest risk of subsequent disease when used prophylactically. These strong effects of prophylactic and therapeutic use of these two classes of antimicrobials suggest that their activity is more potent than other classes. In our study farms, these two classes had a comparatively low level of usage both in terms of frequency and amount (Cuong et al., 2019). We could speculate that these low levels of usage may have selected very little resistance in the microbiota of our study population, making these classes of antibiotics more potent than the others. Demonstrating this, however, is not easy given the vast range of potential pathogens and commensal organisms that may be present in flocks.

In addition to bacterial infections, other possible causes of diarrhoea in poultry include coccidiosis, helminths, viruses (such as rotavirus and adenovirus). Antimicrobials will not treat these non-bacterial pathogens but these products might help to prevent superinfections. Indeed, the pathogens

listed above tend to damage the chicken intestine which allows harmful bacteria to grow out of control in the intestine, leading to a secondary bacterial diarrhoea, increasing the disease severity and ultimately the risk of death.

Leg problems may be caused by a range of aetiologies including bacterial, viral diseases as well as metabolic and nutritional disorders. The observed findings indicating that AMU results in an increase in leg disease is consistent the involvement of non-bacterial pathogens such as Marek virus (leg paresis) and reovirus (viral arthritis with severe lameness and swollen hock) or metabolic/nutritional disorders in the aetiology of these problems.

For episodes of respiratory and CNS diseases, there was not a clear association between therapeutic AMU and mortality, suggesting that, in our setting, primarily non-bacterial pathogens may be responsible for respiratory and CNS infections (i.e. avian influenza, Newcastle, infectious bronchitis, infectious laryngotracheitis, fowlpox, etc.). In the case of respiratory diseases, complex bacterial-viral-vaccine interactions and common, and therefore AMU may not contribute to mitigate the mortality outcome. A recent study has demonstrated the diverse number of viral pathogens that typically affect chickens with respiratory disease in the area (BichVan et al., 2019; Choisy et al., 2019).

The optimal study design to compare the effect of prophylactic AMU would be a randomized controlled trial. However, there are important reasons against such experiment (i.e. high cost, ethical consideration since it involves administering healthy animals with antimicrobial products at large scale). The advantage of the emulation approach is that if the emulation is successful, the analysis of the observational data yields the same effect estimates as the target randomized trial. Inability to control all of eligibility criteria as in a randomized may result in wrong causal inferences. In this study, all potential confounding variables have been add to the model, but stills others need to be considered (i.e. breed of chicken, farming condition etc.). With more than 5,500

observation weeks, this is the first study showed that prophylactic AMU did not reduce the risk of diseases.

To our knowledge, this is the first epidemiological study addressing the impact of prophylactic and therapeutic AMU on the health status of chicken flocks from a low- and middle-income country. The approach we used to define prophylactic and therapeutic AMU (with a pre-selection of weeks) was possible because of the high volume of data collected on a weekly basis (>5,000weeks). A structural limitation of the data is that when both AMU and clinical signs were reported on the same week for the first time in a flock, it was not possible to determine which of the two events occurred first. Because of this, about 50% of the data were excluded, thus decreasing the statistical power of the study. In addition, in most cases, antimicrobial products included two or more AAIs, and disease episodes presented with a combination of different clinical signs. Given the large number of combinations possible, we restricted our analyses to examining the impact of AMU by class on individual clinical signs. Our study also excluded any antimicrobials present in feed as AGPs. This is the case for about 40% of the feed formulations examined. However, the concentrations (strength) of antimicrobials included in these feeds is much lower than the ones used prophylactically. The reason why we did not attempt to measure AGP consumption was that farms often use different feed formulations simultaneously and the labelling is ambiguous (e.g. this feed product may include one of the following antimicrobials: A, B, or C). As many other countries worldwide, Vietnam is also currently engaged in legislative efforts leading to progressive reductions of antimicrobials. In Vietnam a recent Decree (13/2020/ND-CP) includes the timeframe for a ban of AMU for prophylactic purposes (including AGPs), with phased bans for different antimicrobials classes: WHO ‘highest’ and ‘high priority’ critically important AAIs to be banned from 2021, highly important AAIs from 2022, important AAIs from 2023 and all other antimicrobial classes from of 2026. We do not know, however, the level of compliance with this upcoming legislation.

Conclusions

We found evidence that prophylactic AMU does not prevent infection and can instead increase the risk of clinical disease in chicken flocks. In general, prophylactic use of lincosamides, penicillins, methenamines, and tetracyclines tend to increase the risk of diarrhoea, and prophylactic use of lincosamides, penicillins, macrolides and amphenicols tend to increase the risk of respiratory infections. Therapeutic AMU of any classes of antimicrobial resulted in an overall increase in mortality. A majority of classes of antibiotics have a strong therapeutic effect in reducing the mortality associated with diarrhoea infections. However, any therapeutic use of antibiotic in case of leg problems tends on the contrary to increase the risk of death. For respiratory and CNS infection, therapeutic AMU appears highly inconsistent and unpredictable, even within a single class of antimicrobials. Lincosamides, methenamines and cephalosporins are the only antimicrobial classes that always decrease the mortality when used therapeutically. Lincosamides, methenamines are also the two classes of antimicrobials that increase the risk of disease the most when used prophylactically.

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Conflict of interest

The authors declare that they have no conflicts of interest in relation to this paper.

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Table 1: Description of the AMU data, including data for prophylactic AMU analysis (No. weeks with prophylactic AMU and number of weeks without AMU), and therapeutic AMU analysis (No. weeks before and after therapeutic AMU). The data are stratified by antimicrobial class (by row). The ranges reflect the variability resulting from different combinations of the tuning parameters of the categorization algorithm.

Classes	raw AMU data		Data for prophylactic effect analysis		Data for therapeutic effect analysis		
	No. flocks N=349 (%)	No. weeks N = 5,566 (%)	No. weeks with prophylactic-AMU (%)	No. weeks with non-AMU (%)	No. disease episodes	No. weeks before therapeutic -AMU	No. weeks after therapeutic-AMU
Aminolycosides	158 (45.3)	367 (6.6)	125-250 (34.1-68.1)	2841-4554 (54.6-87.6)	44-100	53-396	0-103
Amphenicols	66 (18.9)	100 (1.8)	51-80 (51-80)	3236-4984 (59.2-91.2)	13-30	44-541	0-35
Cephalosporins	7 (2.0)	9 (0.2)	2-8 (22.2-88.9)	3412-5139 (61.4-92.5)	0-1	298-623	0-4
Diaminopyrimidines	56 (16.0)	106 (1.9)	33-74 (31.1-69.8)	3258-4978 (59.7-91.2)	18-34	191-568	0-29
Lincosamides	24 (6.9)	33 (0.6)	15-30 (45.5-90.9)	3367-5093 (60.9-92)	4-9	197-607	0-9
Macrolides	137 (39.3)	310 (5.6)	117-220 (37.7-71)	2909-4638 (55.3-88.2)	22-68	45-478	2-53
Penicillins	113 (32.4)	208 (3.7)	95-160 (45.7-76.9)	3023-4791 (56.4-89.4)	34-61	145-498	0-57
Pleuromutilins	1 (0.3)	1 (0.0)	1-1 (100-100)	3419-5154 (61.4-92.6)	0-0	0-0	0-0
Polypeptides	252 (72.2)	605 (10.9)	261-407 (43.1-67.3)	2249-4158 (45.3-83.8)	63-133	37-340	0-109
Quinolones	98 (28.1)	168 (3.0)	74-129 (44-76.8)	3113-4870 (57.7-90.2)	20-39	42-538	2-50
Sulfonamides	83 (23.8)	148 (2.7)	65-109 (43.9-73.6)	3138-4906 (57.9-90.6)	22-50	46-527	1-52
Tetracyclines	258 (73.9)	628 (11.3)	266-432 (42.4-68.8)	2223-4117 (45-83.4)	57-127	43-339	0-136
Methenamines	26 (7.4)	36 (0.6)	15-31 (41.7-86.1)	3356-5091 (60.7-92.1)	7-16	201-619	0-1
Any class	296 (84.8)	1,266 (22.7)	353-686 (27.9-54.2)	1564-3251 (36.4-75.6)	144-310	21-164	1-153

Antimicrobial agents within each class: Aminolycosides: neomycin, gentamicin, streptomycin, spectinomycin, apramycin, josamycin. Amphenicols: florfenicol, thiamphenicol, chloramphenicol. Cephalosporins: cefadroxil, cefotaxime, cephalexin, ceftiofur.

Diaminopyrimidines: trimethoprim. Lincosamides: lincomycin. Macrolides: tylosin, tilmicosin, erythromycin, spiramycin, kitasamycin. Penicillins: amoxicillin, ampicillin. Pleuromutilins: tiamulin. Polypeptides: colistin, enramycin. Quinolones: enrofloxacin, flumequine, norfloxacin. Sulfonamides: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadimidine, sulfaguanidin, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulphamethoxazole, sulphathiazole. Tetracyclines: oxytetracycline, doxycycline, tetracycline. Methenamines: methenamine.

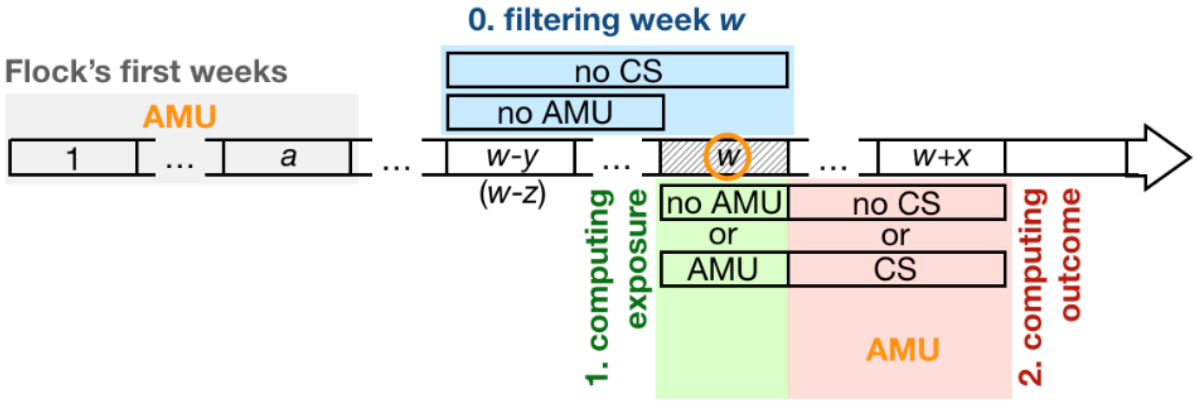


Figure 1: Data preparation for the estimation of the prophylactic effect of AMU. The horizontal arrow represents the time line of a flock, divided into weeks, represented by rectangles, starting on week 1 (on the left). For any given week *w* selected (by step 0, see below) for the analysis (represented here by the hashed rectangle), we computed (i) an exposure variable based on the use or not of antimicrobials (step 1, in green) and (ii) an outcome variable based on the occurrence or not of clinical signs over an observation period of *x* weeks after week *w* (step 2, in red). Statistical analyses then tested whether AMU on week *w* (exposure) affects the occurrence of clinical signs over the observation period (outcome). In order to make sure that AMU exposure on week *w* does correspond to prophylactic AMU, we filtered out all the weeks that were preceded by (i) the presence of clinical signs over a period of *y* weeks before week *w* (including week *w*), or (ii) AMU over a period of *z* weeks before week *w* (naturally excluding the candidate week, since this information is used to compute the exposure variable). This step 0 is shown in blue on the figure. Finally, the analysis includes potential confounding factors (shown in orange letters and circle) such as the age of the chicken (i.e. week *w*) as well as AMU during the first *a* weeks of the flock's life (brooding period, in grey) and during the *x* weeks of the observation period.

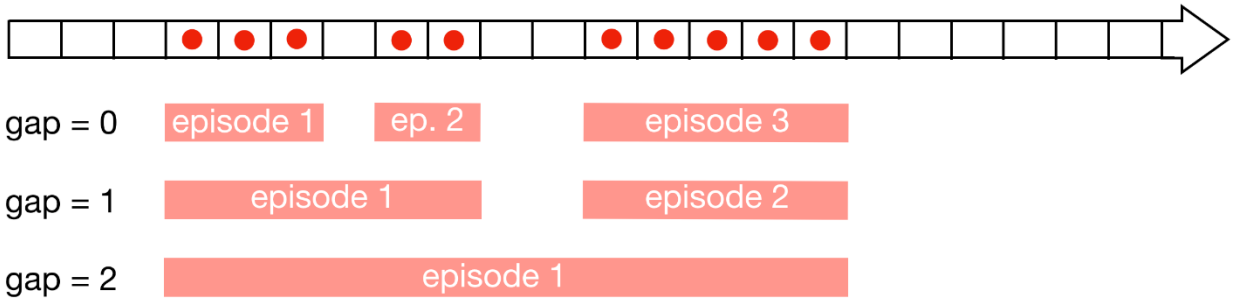


Figure 2: Defining a disease episode. The horizontal arrow represents the time line of a flock, divided into weeks, represented by rectangles, from the first week on the left to the last one on the right. The red dots represent the reporting of disease (clinical sign). In order to account for the fact that clinical signs may not be always reported, we allow the possibility to convert one or a few consecutive weeks without reported clinical signs and surrounded by weeks with reported clinical signs into one single disease episode. The gap parameter is the number of consecutive week(s) without clinical signs we allow when defining a disease episode. Below the time line arrow are 3 examples of disease episodes definitions: 3 episodes when maximum gap = 0 (top), 2 episodes when maximum gap = 1 (middle) and 1 episode only when maximum gap = 2 (bottom).

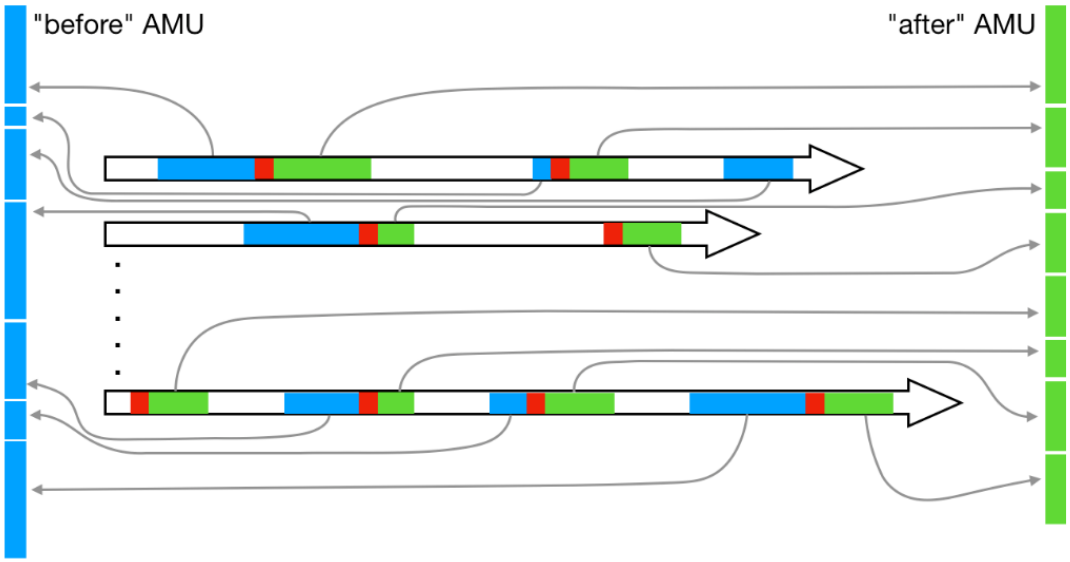
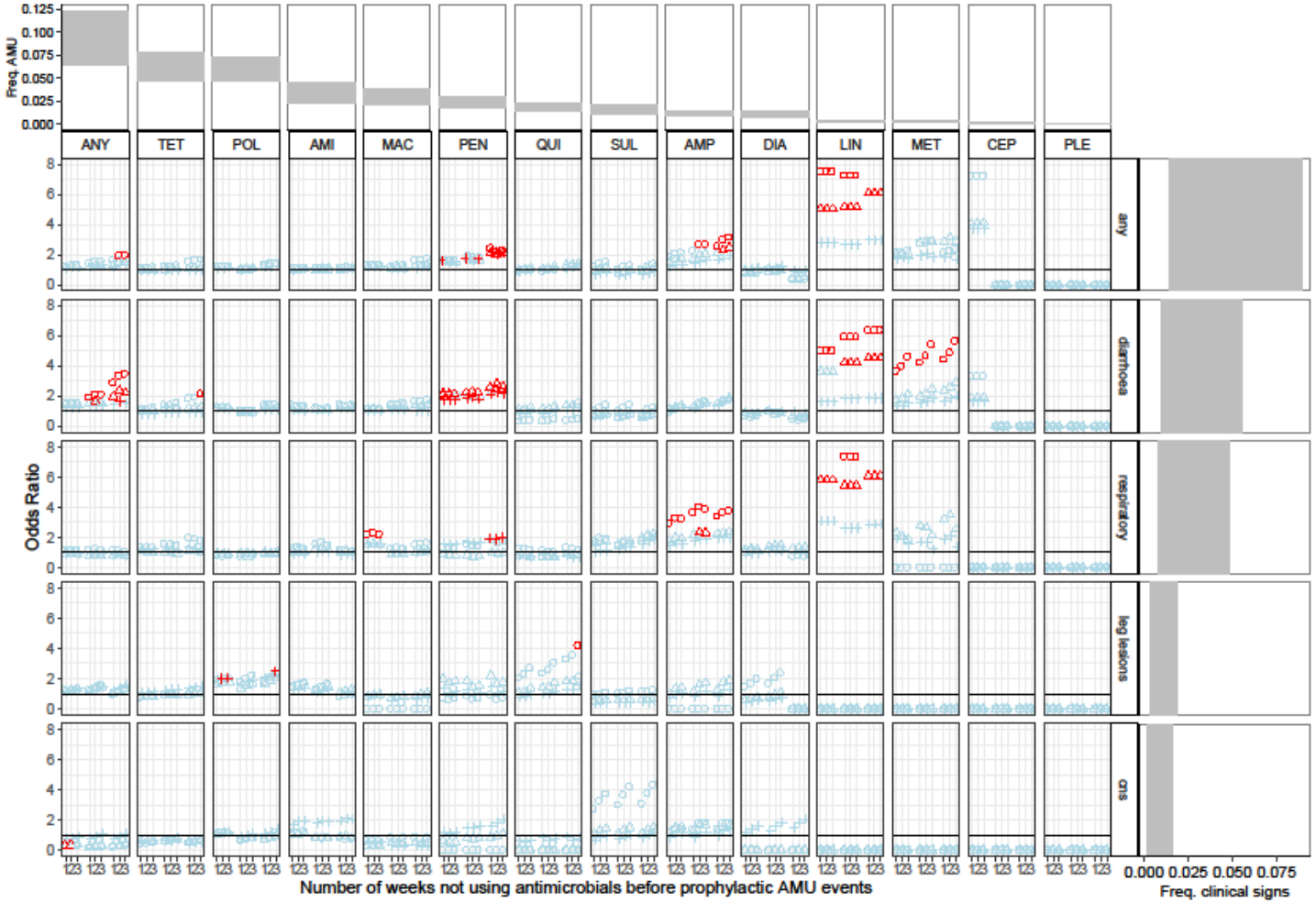


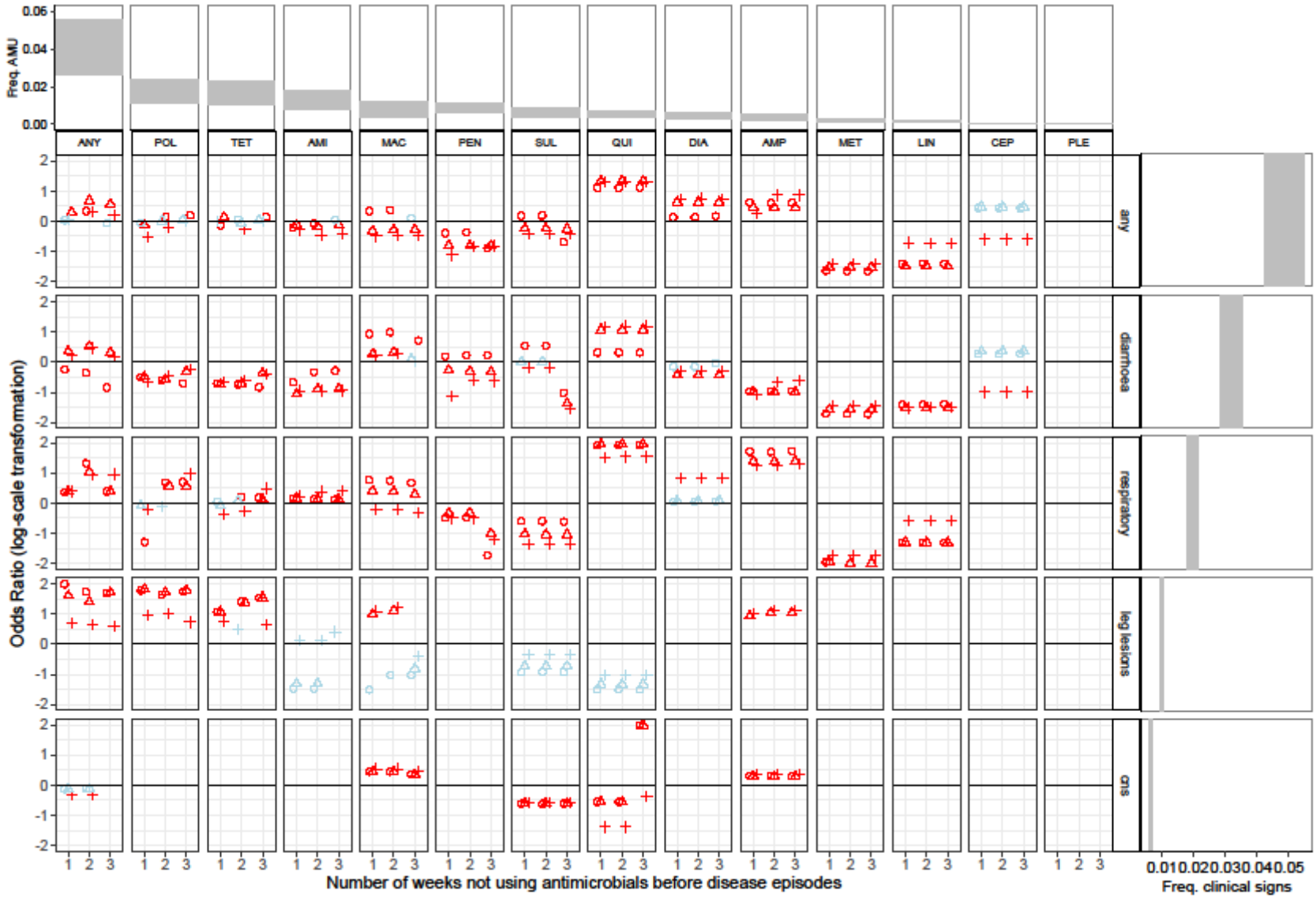
Figure 3: Separating 'before-AMU' and 'after-AMU' arms in all the disease episodes. In this example, the horizontal arrows show the first 2 (top) and the last (bottom) flocks of the data set. Each flock starts on the left end and ends on the right end of the arrow and the length of the arrow is the duration of the flock. Coloured sections represent disease episodes as identified on Figure 2. The red rectangles represent the first week of AMU (if any) in the disease episodes. Sometimes there is no AMU at all during the disease episode (as on the third episode of the first flock) and some other times the first week of AMU is the first week of the episode (as on the second episode of the second flock or the first episode of the last flock). Once these first weeks of AMU are identified in all the disease episodes, we gathered, from all the disease episodes of all the flocks, all the weeks that occur before (in blue) in one arm "before", and all the weeks that occur after (in green) these first weeks of AMU in another arm "after".


Freq AMU
0.125
0.100
0.075
0.050
0.025
0.000
ANY
TET
POL
AMI
MAC
PEN
QUI
SUL
AMP
DIA
LIN
MET
CEP
PLE
any
diarrhoea
respiratory
leg lesions
cns
Odds Ratio
0
2
4
6
8
123
Number of weeks not using antimicrobials before prophylactic AMU events
0.000 0.025 0.050 0.075
Freq. clinical signs

1

2

Figure 4: OR (Odds Ratios) of occurrence of clinical signs when antimicrobials were used prophylactically. For the ease of visualization, confidence intervals are not represented. Instead, red colour indicates OR values that are statistically significant ($p<0.05$) and light blue colour indicates OR values that are not significant. Circle, triangle and cross shapes represent durations *x* of the observation period equal to 1, 2 and 3 weeks respectively. Numbers 1, 2 and 3 represent the duration $y = z$ of the filtering period (the number of weeks without any AMU before prophylactic events). In each subpanel, each combination of three numbers 123 represented, from left to right, the AMU in the first 1, 2 and 3 weeks of life. The horizontal black line represents an OR value of 1. OR values higher than the horizontal black line indicate that the prophylactic AMU increases the risk of having clinical signs. A linear scale instead of a logarithm one was chosen for the OR in order to show the spread of significant values better. Antimicrobial classes were ordered from the most to the least commonly used. Abbreviations: 'ANY' = any classes, 'AMI' = aminoglycosides, 'AMP' = amphenicols, 'CEP' = cephalosporins, 'DIA' = diaminopyrimidines, 'MAC' = macrolides, 'MET' = methenamines, 'LIN' = lincosamides, 'PLE' = pleuromutilins, 'POL' = polypeptides, 'QUI' = quinolones, 'SUL' = sulfonamides, 'TET' = tetracyclines, 'Freq. AMU' = Frequency AMU, 'Freq. clinical signs' = Frequency clinical signs. First row and right column respectively show the ranges of frequencies of AMU and clinical signs observed in the study farms.


Freq AMU
0.06
0.04
0.02
0.00
ANY
POL
TET
AMI
MAC
PEN
SUL
QUI
DIA
AMP
MET
LIN
CEP
PLE
any
diarrhoea
respiratory
leg lesions
ons
Odds Ratio (log-scale transformation)
Number of weeks not using antimicrobials before disease episodes
Freq. clinical signs

1

Figure 5: OR (Odds Ratio) of the impact of therapeutic AMU on mortality. For the ease of visualization, confidence intervals are not represented. Instead, red colour indicates OR values that are statistically significant ($p<0.05$) and light blue colour indicates OR values that are not significant. Cross, circle, triangle shape represented 0, 1, and 2 weeks of gap in disease episodes respectively. The horizontal black line represents an OR of 1. OR values higher than the horizontal black line indicate that therapeutic AMU increases the mortality rate. Antimicrobial classes were ordered from the most to the least commonly used. Abbreviations: 'ANY' = any classes, 'AMI' = aminoglycosides, 'AMP' = amphenicols, 'CEP' = cephalosporins, 'DIA' = diaminopyrimidines, 'MAC' = macrolides, 'MET' = methenamines, 'LIN' = lincosamides, 'PLE' = pleuromutilins, 'POL' = polypeptides, 'QUI' = quinolones, 'SUL' = sulfonamides, 'TET' = tetracyclines. First row and right column respectively show the ranges of frequencies of AMU and clinical signs observed in the study farms.

Chapter 7

Feasibility study of a field survey to measure antimicrobial usage in humans and animals in the Mekong Delta region of Vietnam

Feasibility study of a field survey to measure antimicrobial usage in humans and animals in the Mekong Delta region of Vietnam

Nguyen Van Cuong[1], Nguyen Phuong Cam Ly[2], Nguyen Thi Bich Van[1], Doan Hoang Phu[1,4], Bach Tuan Kiet[3], Bo Ve Hien[3], Pawin Padungtod[6], Guy Thwaites[1,7], Marc Choisy[1,5], Juan Carrique-Mas[1,7,*]

[1] Oxford University Clinical Research Unit, Vietnam

[2] University of Alberta, Canada

[3] Sub-Department of Animal Health and Production (SDAHP), Cao Lanh, Dong Thap, Vietnam

[4] Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, HCMC, Vietnam

[5] MIVEGEC, IRD, CNRS, University of Montpellier, France

[6] Food and Agriculture Organization of the United Nations, Ha Noi, Vietnam

[7] Centre for Tropical Medicine and Global health, Nuffield Department of Medicine, Oxford University, Oxford, United Kingdom

*** Correspondence:**

Juan J Carrique-Mas, DVM, MSc, PhD, MRCVS

Centre for Tropical Medicine

Oxford University Clinical Research Unit

764 Vo Van Kiet, Ward 1, District 5

Ho Chi Minh City (Vietnam)

Abstract

Antimicrobial resistance (AMR) has a particularly high impact on rural human and animal populations in low- and middle-income countries (LMICs). The development of surveillance systems for AMU is a priority action point, but data collection is challenging; an additional complication is the diversity of animal species and metrics. The Mekong Delta region of Vietnam has a high density of human populations and numbers of small-scale farming systems, with a high prevalence of resistance in animal commensal and foodborne pathogens. We collected AMU data from human residents and animals raised in 101 small-scale farms in the Mekong Delta over a fixed period (last 90 days in humans, last 7 days in animals). The aims were to measure and quantitatively compare AMU in humans and the four most common animal species (chickens, ducks, Muscovy ducks, pigs) using different metrics (standing population, 'biomass' and 'Population Correction Unit') and use these estimates to infer consumption data for the Mekong Delta region of Vietnam. Humans used 5.9 DDD_{kg} (SD ± 18.0), or 273.3 mg of AAIs (SD ±880.8) per kg standing bodymass per year; animals consumed 90.0 ADD_{kg} (SD ±141.9) or 3,011 mg (SD ±7,431) of AAIs per kg standing bodymass per year. Individuals <5 and >65 years-old consumed significantly more than people in other age categories. For the estimation of total antimicrobial usage for the Mekong Delta region, pigs were the target of the greatest amounts of AMU, both in terms of frequency (40.6%) and quantity (36.1%). Over one year humans consumed a total of 122.2 tonnes, or 26.2% of total AMU. However, per kg of body weight, Muscovy ducks were the target of the greatest amounts of AMU and human consumed less than 5% of total AMU in all metrics. AAIs regarded of critical importance by WHO in animals (71.8% No. ADD_{kg}) were considerably higher magnitude compared

with humans (47.4% No. DDD_{kg}). In general, for animal AMU, metrics that related AMU to standing bodymass were higher than those relating AMU to biomass or PCU. Using a One Health approach, we demonstrated that accurate AMU can be estimated from a simple cross-sectional surveys, although results are very sensitive to the chosen metric. Results confirm the preponderance of AMU in animal populations. This methodology can potentially be applied in AMU surveillance in low-resource settings, allowing to focus reduction efforts AMU in particular animal species.

Keywords: Antimicrobial use, farmer, animal, small-scale farms, Mekong Delta, One Health, Vietnam.

Introduction

The global crisis of antimicrobial resistance (AMR) has a particular severe impact on rural human and animal populations of low- and middle-income countries (LMICs) due to limited medical and veterinary care resources [1, 2]. The presence of high densities of mixed species, small-scale farming systems, alongside excessive levels of antimicrobial use (AMU) in many of these areas contribute to the problem [3]. There is now considerable evidence of a link between excessive antimicrobial use (AMU) and the occurrence of resistance in humans and animals [4, 5].

The Tripartite Global Action Plan on AMR, jointly developed by WHO, FAO and OIE [6], has established that AMU surveillance is a key priority action point under strategic Objective 2. However, many LMICs countries lack adequate systems for AMU surveillance due to limited resources and capacity to collect AMU data. The World Organization for Animal Health (OIE) compiles annual surveillance reports of AMU in animal production globally [7]. However, these reports do not attempt to compare AMU between different species or production types, a necessary calculation in order to identify those production types where AMU/AMR is likely to be highest (i.e. hotspots). Furthermore, beyond limited research studies, there are no official global data on AMU in human community populations [8].

The selection of metrics for quantification of AMU is crucial, since different metrics can lead to considerable differences in estimates [9-11]. In its annual global report, the OIE relates quantities of antimicrobials (in mg) to the weight (biomass) of all animals produced, expressed as a sum of the bodyweight of slaughtered animals plus that of standing animals [7]. In contrast, the European Union (EU) in its Annual ESVAC reports relate weigth of

AAI to animal populations computed using Population Correction Units (PCU), which corresponds to the typical treatment weight for each of the standing and slaughtered animal species [12].

A recent study estimated total AMU related to animal and human biomass in Vietnam. The animal population denominator was calculated following similar methodology to that adopted by the OIE in its annual report [13]. The study concluded that overall, 262mg and 247mg of antimicrobial active ingredients (AAIs) were, respectively used per kg of animal and human biomass across the country. However, results were largely based on extrapolations from previous surveys on a limited number of species and locations, not on real survey data. More accurate species-specific information is required to properly production types where the selection pressure on AMR is greatest. The accurate estimation of AMU in humans and animals present considerable logistic challenges since this requires conducting longitudinal studies, which are both laborious and costly.

The Mekong Delta region, located in the southwest region of Vietnam (40,500 km^2 and 17.8M population in 2019) is regarded as a hotspot of AMU and AMR in animal production. Previous studies conducted in this predominantly rural area have evidenced a high prevalence of AMR in commensal *Escherichia coli* from chickens, ducks, pigs [14, 15] as well as from poultry farmers [16, 17]. Similarly, food-borne pathogens (i.e. non-typhoidal *Salmonella* and *Campylobacter* spp.) isolated from pigs and poultry farms also display resistance against a large number of antimicrobials [18, 19]. Unsurprisingly, the quantities of antimicrobials used in chicken and pig farms in the area are high [10, 20]. However, in addition to chickens and pigs, many small-scale farms typically raise a range of species including fish, ducks, Muscovy ducks, and to a lesser extent, ruminants (cattle

and goats). The relative intensity of AMU in different animal species and humans has yet to be determined in the area and in Vietnam as a whole. Most owners of small-scale farms make decisions regarding AMU based on their own knowledge and experience [21].

Using a cross-sectional, One Health study approach, we quantified AMU in human residents and animals raised in smallholder farms in the Mekong Delta region of Vietnam. The aims were: (i) to describe and compare the types and quantities of antimicrobials used by animals and humans; (ii) to extrapolate these AMU results to the Mekong Delta of Vietnam using different denominator units; and (iii) to compare levels of AMU in different animal species, as well as between animals and humans. This study should provide insights to help design AMU surveillance systems, whilst clarifying the implication of the choice of denominator units.

Materials and Methods

Study design

We conducted a cross-sectional survey of poultry-raising households located in 5 of the 12 districts of Dong Thap province (Mekong Delta, Vietnam) during July 2019. The five districts were chosen based on convenience to be located less than 30km from the provincial capital. We requested the veterinary authority in each district (District Veterinary Station, DVS) for a list of livestock farms of typical size and mix of species raised. From each district, 20-25 small-scale poultry farms (defined as raising chickens and/or ducks for commercial purposes, i.e. not for family consumption) were selected by the DVS veterinarian, and selected farm owners were invited to join the study. We aimed to sample ~100 households. All visits were conducted by staff affiliated to the Sub-Department of Animal Health and Production of Dong Thap (SDAHP-DT) during July

2019. In each selected household, the person identified as being responsible for taking care about family members and animals the most was interviewed. The data collected, that included the identity of specific AAIs consumed and the frequency (days) was used to calculate AMU, both in terms of doses (treatment intensity) and quantities (weight of AAI) for the province and the Mekong Delta region by species.

Data collection

A structured questionnaire was developed to gather data on household farm human residents (age, gender, education, occupation, degree of contact with animals), as well as on numbers and age of food animals (i.e. excluding companion animals, frogs, ornamental birds and fish) present in the farm on the visit date (Appendix 1). To minimize recall bias, we enquired about administration of antimicrobials over a period consisting of the latest 90 and 7 days (for humans and animals, respectively). In all visits, the investigators requested to inspect the cabinets used to keep medicines of humans and animals. The examination of the cabinets was taken as an opportunity to discuss information on types, doses, and concentration of any antimicrobials identified in the cabinets. All medicine products containing antimicrobial active ingredients (AAIs) were singled out after reviewing the label or the prescription (for human medicines). AAI were classified based on the WHO list of antimicrobial agents [22]. The concentration of AAIs contained in these products was described and was expressed in mg/tablet (human products) and mg or ml per kg of product (animal products). Those veterinary antimicrobials not appearing in the WHO list were classified according to the OIE list of antimicrobial agents [23].

Quantification of AMU in the surveyed population

We first estimated annual consumption in the surveyed population using frequency-based metrics: No. of defined daily doses kg (DDD_{kg}) for humans, and No. of Animal Daily Doses kg (ADD_{kg}) for animals. This was achieved by multiplying the reported number of days when antimicrobials were consumed over the observed period (90 and 7 days for humans and animals, respectively) by the estimated bodymass (in kg) of each treated person/flock/herd. These frequency estimates were also related to the 'standing bodymass' of the surveyed population as well as to 'bodymass-time' denominator in order to compute the 'treatment intensity'. The resulting estimates were averaged for each species. For example, consumption of ampicillin over 2 days (out of 90 days) by an 80kg person equates to consumption of 160 DDD_{kg} (80*2), this equates to 160*(365/90)=648.9 DDD_{kg} per year, or to 648.9/80=8.1 DDD_{kg} per kg of bodymass. The body-mass time denominator for that individual would be 365*80=29,200 (kg-days), therefore the treatment intensity (equivalent to a daily probability of consuming antimicrobials) for that individual would be 648.9/29,200=0.022. Similarly, in a flock of 20 chickens (each weighing 2kg) where 10 chicken were administered ampicillin for 4 days over a 7-day period, consumption for the flock was estimated in 80 ADD_{kg} (2*4*10). Over a year, this flock would be expected to consume 4,170 ADD_{kg} (80*365/7). The bodymass time denominator for that flock is 365*2*20=14,600 kg-days. This flock would have consumed 104.2 ADD_{kg} (4,170/40) per kg of bodymass per year, and the treatment intensity would be 0.286 (170/14,600).

For humans, the quantities (weight) of AAIs consumed were estimated from the actual doctors' prescriptions where available. For animals, they were inferred from frequency data and the preparation instructions of each product consumed. The estimated number of ADD_{kg} was multiplied by the 'technical dose' of each of the AAI consumed. The 'technical

dose' values for each AAI were inferred from the antimicrobial products' preparation instructions (as written in the label) and from the prescription data in the case of human antimicrobial products. For animals, the 'technical ADD_{kg}' was defined as 75% of the 'treatment' daily dose for each AAI for 1 kg of live animal bodyweight, and was obtained from the preparation instructions as indicated in the products' labels and consumption estimated from the weight (inferred from age) of the animal. Animal weight, feed and water consumption for each animal species by age (in weeks) were inferred from previous studies [10, 24, 25]. Respectively, 0.225 l and 0.120 l was taken as the daily water intake, and 0.063kg and 0.037kg as the daily consumption of feed of a 1 kg bird (any species) and and pig. For injectable preparations (animal products), the preparation instruction (i.e. 5ml/kg/day) were used to estimate the consumption of AMU.

In cases where participants stated consumption of AAI, but did not remember for how many days, we assigned the average of the remaining observations. To account for individuals that reported using medicine, but did not remember whether it included AAI or not, we assumed that a fraction of them equivalent to the ratio of AAI to medicine consumed AAI. For individuals where AAI concentration or dosage data were missing, we extrapolated from similar products in the same species. Human medicine products administered to animals flocks were excluded from the estimation of ADD_{kg} values since the guidelines for preparation for animal used were not available.

Estimations of AMU in the Mekong Delta of Vietnam

AMU data by species obtained from the survey was extrapolated for human and animal populations in the Mekong Delta region (2019). The data were presented using four different metrics: (i) No. doses-kg (either DDD_{kg} or ADD_{kg}) related to the total amount of

kg-bodymass-time for each species per year; (ii) Weight of AMU related to 'standing bodymass' of human and animal populations per year; (iii) Weight of AMU related to 'standing bodymass' of human and 'biomass' of animal populations per year (i.e. OIE method) [26]; (4) Weight of AMU related to 'standing bodymass' of human and 'PCU' of animal populations per year (i.e. ESVAC method) [27].

The human 'standing bodymass' was obtained by multiplying the number of individuals (census) by their bodyweight (estimated from their age and gender) [28, 29]. To estimate animal 'standing bodymass', the number of animals of each species (from census data) [30] was multiplied by their estimated bodyweight of each species in their mid-age [13]. To estimate 'biomass' of animals, the number of animals produced (from production data) of each species was multiplied by the total individual animal bodyweight at slaughter time. The number of 'Population Correction Units' (PCU) for each animal species was calculated as the total number of animals multiplied by the average weight at treatment based on ESVAC guidelines. Detailed calculations are presented in Appendix 2 (Table 1 and 2).

Ethics

Ethics approval for the study was obtained from the Oxford Tropical Research Ethics Committee (OxTREC), Oxford, UK (Reference No. 533-19). Informed consent was obtained from all participants.

Results

Participants and household farm characteristics

A total of 101 household farms with 316 human residents that were present in the household at the time of the farm visit were investigated. Their sociodemographic characteristics are displayed in **Supplementary Material 1.** Each farm had a median of 3

residents [Inter-quartile range (IQR) 2-4], with a median age of 38 [IQR 13-55] years. Over half (51.9%) were males. All interviewees were above 18 year-old, and 82.2% were male. A total of 186 (58.8%) residents reported direct contact with animals in their farms.

Of 101 farms investigated, chickens were the predominant species (71.3% farms), followed by ducks (54.5%), pigs (19.8%), Muscovy ducks (11.9%), fish (10.9%), cattle (5.9%), frogs (4.0%), goats (2.0%), geese and eels (1.0% each). Farms raising only one food animal species (42.6%) were predominant, followed by farms raising two (40.6%), three (11.9%), five (2.9%) and four (1.9%) species. The most common species combinations were 'chicken-duck' (17.8%), followed by 'chicken-Muscovy duck' (4.9%), 'chicken-duck-pig' (4.9%), 'chicken-pig' (3.9%) and 'duck-pig' (3.9%). The median chicken flock age was 12 weeks [IQR 4-24] and the median flock size was 30 [IQR 15-75]. For duck farms, the median flock age was 8 weeks [IQR 4-25], and a median flock size of 100 [IQR 40-700]. The median pig age was 15 weeks [IQR 8-51], with a median herd size of 10 [IQR 3-12]. Details on all species present in study farms is given in **Supplementary Material 2**.

AMU in humans and animals in the farms surveyed

A total of 173/316 participants (54.7%) reported using medicine over the last 90 days. However, only 121 participants (38.2%) kept their records or doctor prescriptions. Forty two out of 121 participants (34.7%) (median age 43 [IQR 7-55.5] years) confirmed using antimicrobial-containing products during the last 90 days. For the 52 participants that consumed medicine, but did not remember whether it was a AAI or not, we assumed that 34.7% used of them actually contained an AAI. Therefore antimicrobial consumption over the last 90 days was assumed for 60 (34.7%) individuals (42+[52*0.347]). AMU was reported in a total of 98/320 (30.6%) animal groups over the 7 days prior to the time of interview. AMU was reported in all farmed species except cattle (6 farms), goats (2) and geese (1). A total of 106 (32 in humans and 74 in animals) different AAI-containing products were identified. All human AAI-containing products had been administered through the oral route, and contained one AAI. Fifty-four % antimicrobial-containing products administered to animals contained two AAIs. Most products (75.6%) were administered through the oral route, the remaining were injectable. A total of 9 and 63 antimicrobial-containing products were used in pig and poultry flocks, respectively. Four products were used both in pig and poultry flocks. In addition, 20 products administered to three animal species (chickens, ducks and pigs) were antimicrobials intended for human use. A summary of all antimicrobial-containing products and their associated 'technical daily doses used' is given in **Supplementary Material 3**). Among products, the average technical dose for humans (DDD_{kg}) was 17.3 mg/kg. For pigs and poultry the average values of the technical dose (ADD_{kg}) were respectively, 11.9 and 12.4 mg/kg for antimicrobials consumed orally, and 7.4 and 9.2 mg/kg for those injected. Among AAIs

used by humans, 3rd generations cephalosporins class has the lowest DDD_{kg} (range 3.2-9.2 mg/kg), whilst penicillins had the highest (range 23.0-26.6 mg/kg). The ADD_{kg} for some AAIs were particulary high, notably doxycycline (29.6 mg/kg) sulfadimidines (63.4 mg/kg), thiamphenicol (24.7 mg/kg) and gentamicin (23.2 mg/kg) intended for poultry administered orally, as well as oral sulfamethoxazole (40.5 mg/kg) intended for pigs. Surprisingly, the average injection dose of some AAIs were higher than the average oral doses (i.e. oxytetracycline, 12.1 vs 6.9 mg/kg; spectinomycin 10.3 vs 1.8 mg/kg). The daily doses of each AAI identified in the survey are summarized in **Table 1.**

Table 1. The average antimicrobials technical dose (TD) (DDD_{kg} or ADD_{kg}) from 32 human and 74 animal products used in 101 study farms (AAI used in animals intended for human use were excluded). CV: Coefficient of variation. Critically important antimicrobial classes according to WHO are highlighted:* High priority, ** Highest priority. [#]AAI used for animal but purchased from human medicine.

Class	AAI	Humans (Oral)		Poultry				Pigs			
				Oral		Injection		Oral		Injection	
		n	TD (±CV)	n	TD (±CV)	n	TD (±CV)	n	TD (±CV)	n	TD (±CV)
Tetracyclines*	Tetracycline[#]	1	23.0 (±NC)	3	12.5 (±53.5)						
	Oxytetracycline			9	6.9 (±87.8)	4	12.1 (±98.5)			1	3.7 (±NC)
	Doxycycline			7	29.6 (±151.8)	1	8.4 (±NC)				
Sulfonamides	Sulfaquinoxaline			2	18.3 (±76.6)						
	Sulfamethoxazole[#]			1	18.7 (±NC)			1	40.5 (±NC)		
	Sulfaguanidine[#]										
	Sulfadimidine			2	63.4 (±66.1)						
	Sulfadimethoxine			1	15 (±NC)						
Quinolones	Norfloxacin			2	8.5 (±104.5)						
	Marbofloxacin										
	Enrofloxacin			6	11.0 (±36.3)	4	5.6 (±47.1)				
	Ciprofloxacin[#]	1	18.4 (±NC)								
	Ofloxacin	1	7.3 (±NC)								
Polypeptides**	Colistin			11	3.1 (±36.3)			2	3.7 (±28.9)		
Penicillins*	Ampicillin	1	23.0 (±NC)	5	8.1 (±82.6)			2	9.3 (±NC)		
	Amoxicillin[#]	10	26.6 (±49.2)	3	13.7 (±28.3)	1	0.78 (±NC)	2	15.3 (±44.8)		
	Penicillin V	1	23.0 (±NC)								
Macrolides**	Tylosin			8	10.0 (±90.5)	2	9.3 (±28.2)			2	11.1 (±129.9)
	Spiramycin	2	7.5 (±32.6)	1	0.45 (±NC)	1	3.0 (±NC)				
	Erythromycin			1	15.0 (±NC)						

Table 1. (cont.)

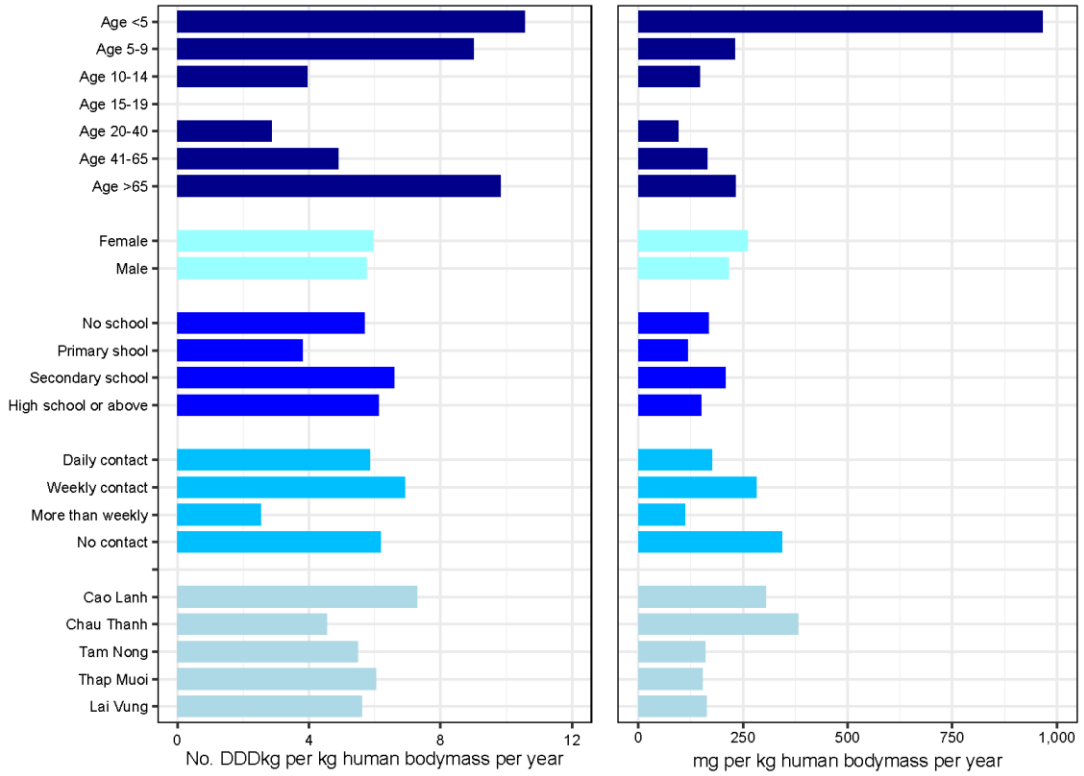
Class	AAI	Humans (Oral)		Poultry				Pigs			
				Oral		Injection		Oral		Injection	
		n	TD (±CV)	n	TD (±CV)	n	TD (±CV)	n	TD (±CV)	n	TD (±CV)
Lincosamides	Lincomycin	1	23.0 (±NC)	1	0.75 (±NC)	3	5.1 (±39.7)				
Diaminopyrimidines	Trimethoprim			3	3.6 (±51.9)			1	8.1 (±NC)		
1st &2nd Cephalosporins	Cefuroxime	5	18.5 (±50.3)								
	Cefotaxime#					1	9.7 (±NC)				
	Cefalexin	4	13.8 (±44.5)	1	7.5 (±NC)						
	Cefdinir	1	13.8 (±NC)								
	Ceftiofur#					1	3.75 (±NC)				
3rd Cephalosporins**	Cefixim	1	9.2 (±NC)								
	Cefpodoxime	2	3.2 (±60.6)								
	Cefadroxil	1	9.2 (±NC)								
Amphenicols	Thiamphenicol			1	24.7 (±NC)	4	16.8 (±55.9)			1	7.5 (±NC)
	Florfenicol			2	5.6 (±120.3)	1	1.8 (±NC)			1	3.7 (±NC)
Aminoglycosides*	Streptomycin			2	9.9 (±34.6)						
	Spectinomycin			1	1.8 (±NC)	4	10.3 (±34.8)				
	Kanamycin			2	8.0 (±75.6)						
	Gentamicin			4	23.2 (±148.6)			2	5.5 (±14.4)		
Average all products		32	17.3 (±61.2)	52	12.4 (±156.4)	17	9.2 (±79.3)	5	11.9 (±99.1)	4	7.4 (±97.2)

Over one year human residents in the farms surveyed were estimated to consume on average 5.9 DDD_{kg} (SD ±18.0) or 237.3 mg (SD ±880.8) per kg of standing bodymass. Averaging across species, animals consumed 90.0 ADD_{kg} (SD ±141.9) or 3,011 mg (SD ±7,431) of AAIs per kg bodymass per year. Consumption among Muscovy ducks was greatest (136.3 ADD_{kg}), followed by ducks (80.3 ADD_{kg}), pigs (73.3 ADD_{kg}) and chickens (70.3 ADD_{kg}). In terms of quantities of AAI related to standing bodymass, Muscovy ducks also consumed the greatest amounts of AAIs (6,436 mg), followed by chickens (2,288 mg), ducks (1,803 mg) and pigs (1,516 mg) **(Table 2).** Individuals aged less than 5 and more than 65 years-old consumed significantly more antimicrobials than people in other age categories (**Figure 1**). There were no significant differences in antimicrobial consumption with respect to other sociodemographic factors such as gender, education, animal contact or district location.

Table 2. Estimations of AMU from the survey of 101 farming households.

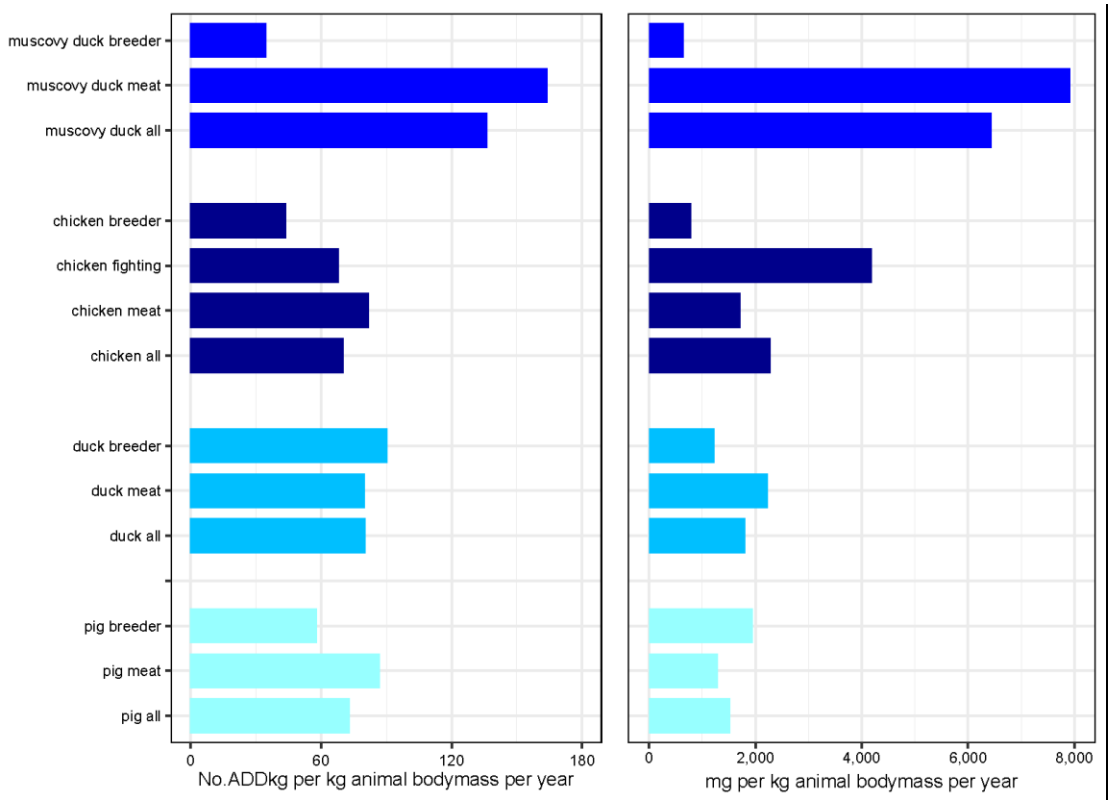
	Humans	Chickens	Ducks	Muscovy ducks	Pigs
No. households	101	72	55	12	20
No. individuals/animals	316	15,933	43,784	1,378	494
Total bodymass (kg)	14,420	16,807	92,280	827	21,145
Total kg-days per year	5,263,300	6,134,555	33,682,200	301,855	7,717,925
Gross AMU					
No. daily doses kg per year	101,909	6,150,487	23,801,832	406,700	1,519,547
mg antimicrobials per year	2,556,235	224,851,445	229,155,140	6,765,427	6,267,780
AMU related to population					
No. doses per kg bodymass-year (mean ± SD)	5.9 (±18.0)	70.3 (±121.2)	80.3 (±144.5)	136.3 (±167.4)	73.3 (±134.6)
mg antimicrobials per kg bodymass-year (mean ± SD)	237.3 (±880.8)	2,288 (±6,317)	1,803 (±4,368)	6,436 (±15,654)	1,516 (±3,387)
Treatment intensity (mean ± SD)	0.015 (±0.048)	0.193 (±0.332)	0.220 (±0.395)	0.373 (±0.458)	0.200 (±0.368)

Figure 1. AMU in human participants by their sociodemographic characteristics.



AMU in the four animal species investigated is displayed in **Figure 2** stratified by production purpose. AMU consumption varied across production types: In general, animals raised for meat consumed more antimicrobials than those raised for breeding purposes. An exception was ducks, where AMU was more frequent in breeding birds than in meat ducks (90.3 ADD_{kg} vs 80.0 ADD_{kg}); in terms of quantities, breeding pig were the target of higher levels of AMU than any other species.

Figure 2. AMU consumption in animal by four species with specific type of production.



Antimicrobial active ingredients (AAIs) used in humans and animals

Table 3 presents frequency data on AAI consumption among humans and animals from the household survey. In humans, 14 different AAIs belonging to 6 different classes were consumed. AAIs categorised as 'of critical importance' by WHO represented 47.4% of the total number of DDD_{kg}. First and 2nd generation cephalosporins were consumed the most (47.8% of total DDD_{kg}), followed by penicillins (39.0%) and quinolones (8.8%). In animals, a total of 30 different AAIs belonging to 11 classes were idetified. AAIs regarded of critical importance by WHO represented 47.4% and 71.8% of the total number of doses given to humans and animals (chickens 64.3%, ducks 65.4%, Muscovy ducks 79.8% and pigs 77.7%), respectively. In terms of frequency, tetracyclines (23.1%), aminoglycosides (12.7%) and sulfonamides (9.9%) classes were the most consumed AAIs by chickens. For pigs, penicillins (50.9%) represented the most frequently used class, followed by quinolones (12.6%). **Table 4** shows AMU in terms of weight of AAI. In humans, the highest quantities of AMU corresponded to the penicillins class (58.0% total), followed by 1st and 2nd generation cephalosporins (39.5%) and 3rd generation cephalosporins (6.8%). In animals, sulfonamides (32.6%), tetracyclines (14.4%), and amphenicols (14.2%) classes were consumed the most by chickens. The highest target for duck and Muscovy duck were tetracyclines (26.9% and 47.0%) and macrolides (18.4% and 22.1%) and. In pigs, penicillin (41.1%) class was used the most frequent, followed by macrolides (30.2%).

Table 3. Estimated annual AAIs consumption expressed in terms of doses per kg of bodyweight (DDD_{kg} in humans and ADD_{kg} in animals) calculated from the small-scale farm survey. The percent is indicated in parenthesis. Critically important antimicrobial classes according to WHO are highlighted: *High priority, **Highest priority. [#]AAIs in products administered to animal populations that were intended for human use.

Class	AAI	Humans	Chickens	Ducks	Muscovy ducks	Pigs
Tetracyclines*	Tetracycline[#]	0.03 (0.5)	1.3 (1.9)	1.4 (1.7)	18.3 (13.4)	
	Oxytetracycline		11.4 (16.3)	10.9 (13.6)	21.3 (15.6)	0.5 (0.6)
	Doxycycline		3.5 (5.0)	9.2 (11.4)	8.2 (6.0)	
	Any tetracyclines	***0.03 (0.5)***	***16.3 (23.1)***	***21.4 (26.7)***	***47.7 (35.0)***	***0.5 (0.6)***
Sulfonamides	Sulfaquinoxaline		1.8 (2.6)			
	Sulfamethoxazole[#]		3.1 (4.4)			0.7 (0.9)
	Sulfaguanidine[#]					
	Sulfadimidine		1.6 (2.2)			
	Sulfadimethoxine		0.5 (0.7)			
	Any sulfonamides		***7.0 (9.9)***			***0.7 (0.9)***
Quinolones	Norfloxacin			3.5 (4.3)	13.0 (9.6)	
	Marbofloxacin		1.4 (2.0)			
	Enrofloxacin		3.7 (5.3)	16.9 (21.1)	5.3 (3.8)	9.2 (12.6)
	Ciprofloxacin[#]	0.39 (6.6)				
	Ofloxacin	0.13 (2.2)				
	Any quinolones	***0.5 (8.8)***	***5.1 (7.3)***	***20.4 (25.4)***	***18.3 (13.4)***	***9.2 (12.6)***
Polypeptides**	Colistin		7.9 (11.3)	14.6 (18.2)	26.5 (19.4)	5.5 (7.5)
	Any polypeptides		***7.9 (11.3)***	***14.6 (18.2)***	***26.5 (19.4)***	***5.5 (7.5)***
Penicillins*	Ampicillin	0.07 (1.3)	1.6 (2.2)	5.7 (7.1)	2.6 (1.9)	2.7 (3.7)
	Amoxicillin[#]	2.15 36.5)	2.9 (4.1)	1.6 (2.0)		34.6 (47.2)
	Penicillin V	0.07 (1.3)				
	Any penicillins	***2.29 (39.0)***	***4.4 (6.3)***	***7.3 (9.1)***	***2.6 (1.9)***	***37.3 (50.9)***
Macrolides**	Tylosin		5.9 (8.4)	5.2 (6.4)	30.4 (22.3)	10.3 (14.0)
	Spiramycin	0.03 (0.5)	1.0 (1.4)	1.4 (1.5)		
	Erythromycin		0.8 (1.1)			
	Any macrolides	***0.03 (0.5)***	***7.7 (10.9)***	***6.6 (7.9)***	***30.4 (22.3)***	***10.3 (14.0)***
Lincosamides	Lincomycin	0.04 (0.6)	5.1 (7.2)	0.3 (0.4)		
	Any lincosamides	***0.04 (0.6)***	***5.1 (7.2)***	***0.3 (0.4)***		
Diaminopyrimidines	Trimethoprim		1.9 (2.7)	1.9 (2.4)	2.6 (1.9)	0.7 (0.9)
	Any diaminopyrimidines		***1.9 (2.7)***	***1.9 (2.4)***	***2.6 (1.9)***	***073 (097)***
1st &2nd gen. cephalosporins	Cefuroxime	1.09 (18.5)				
	Cefotaxime[#]			1.4 (1.7)		

	Cefalexin	1.34 27.3)	1.6 (2.2)			
	Cefdinir	0.09 (2.0)				
	Any 1st &2nd gen. cephalosporins	***2.5 (47.8)***	***1.6 (2.2)***	***1.4 (1.7)***		
	Ceftiofur#			0.9 (1.2)		
	Cefixim	0.17 (2.9)				
3rd Cephalosporins**	Cefpodoxime	0.17 (2.9)				
	Cefadroxil	0.09 (1.6)				
	Any 3rd cephalosporins	***0.43 (7.4)***		***0.9 (1.2)***		
Amphenicols	Thiamphenicol		4.1 (5.9)	2.6 (3.2)		0.5 (0.6)
	Florfenicol		0.3 (0.4)	0.2 (0.3)		5.1 (7.0)
	Any amphenicols		***4.4 (6.3)***	***2.8 (3.5)***		***5.6 (7.6))***
Aminoglycosides*	Streptomycin		1.8 (2.6)	0.3 (0.4)		
	Spectinomycin		5.1 (7.3)	0.3 (0.4)		
	Kanamycin		0.1 (0.2)	0.7 (0.9)		
	Gentamicin		1.8 (2.6)	1.4 (1.7)	8.2 (6.0)	3.4 (4.7)
	Any aminoglycosides		***8.9 (12.7)***	***2.8 (3.5)***	***8.2 (6.0)***	***3.4 (4.7)***
Grand total		***5.9 (100)***	***70.3 (100)***	***80.3 (100)***	***136.3 (100)***	***73.3 (100)***

Table 4. Estimated annual AMU expressed in terms of weight (mg) of AAI as calculated from the farm survey. The percent is indicated in parenthesis. Critically important antimicrobial classes according to WHO are highlighted: *High priority, **Highest priority. #AAIs in products administered to animal populations that were intended for human use.

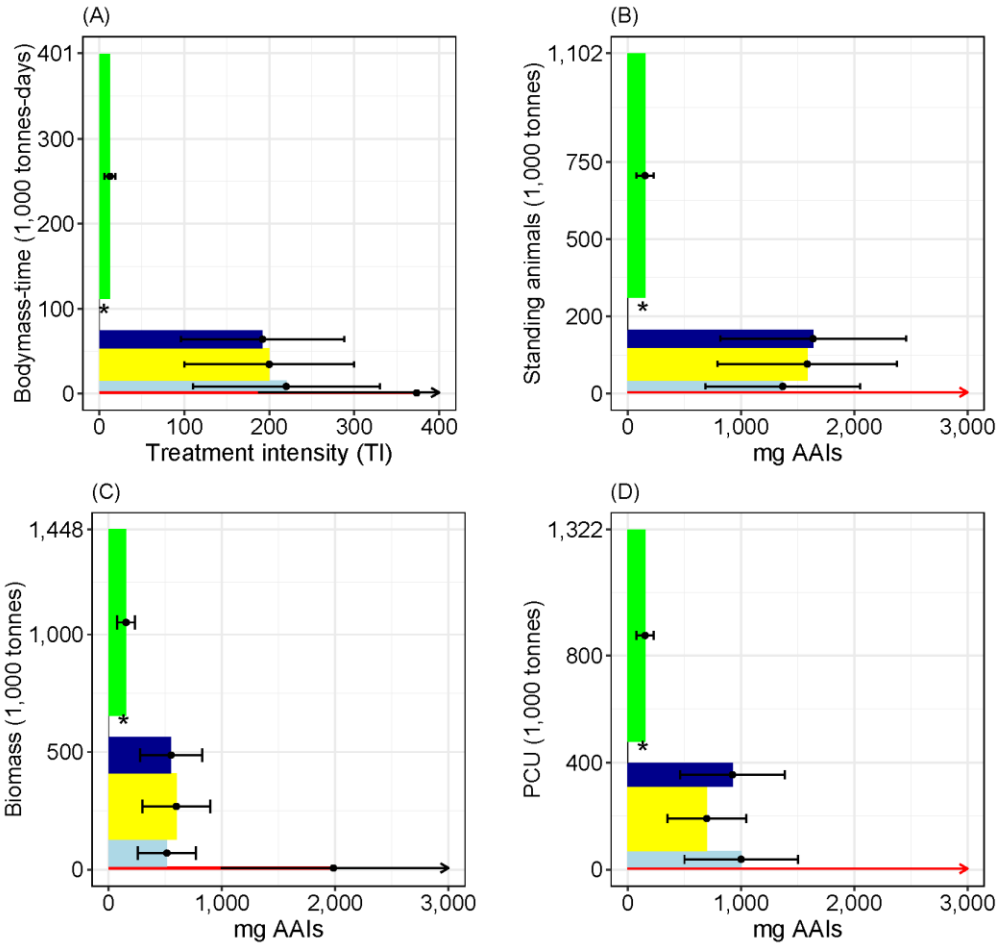
Class	AAI	Humans	Chickens	Ducks	Muscovy ducks	Pigs
Tetracyclines*	Tetracycline#	3.1 (1.3)	51.1 (2.2)	34.4 (1.9)	684.4 (10.6)	
	Oxytetracycline		162.1 (7.1)	147.9 (8.2)	190.4 (3.0)	3.4 (0.3)
	Doxycycline		115.5 (5.0)	302.0 (16.7)	2,150.9 (33.4)	
	Any tetracyclines	***3.1 (1.3)***	***328.7 (14.4)***	***484.3 (26.9)***	***3,025.7 (47.0)***	***3.4 (0.3)***
Sulfonamides	Sulfaquinoxaline		155.5 (6.8)			
	Sulfamethoxazole#		58.2 (2.5)			55.6 (3.7)
	Sulfaguanidine#					
	Sulfadimidine		517.7 (22.6)			
	Sulfadimethoxine		15.5 (0.7)			
	Any sulfonamides		***746.7 (32.6)***			***55.6 (3.7)***
Quinolones	Norfloxacin			7.8 (0.4)	195.5 (3.0)	
	Marbofloxacin		9.4 (0.4)			
	Enrofloxacin		43.5 (1.9)	299.2 (16.6)	97.8 (1.5)	92.6 (6.1)
	Ciprofloxacin#	6.6 (2.7)				
	Ofloxacin	0.9 (0.4)				
	Any quinolones	***7.4 (3.1)***	***52.9 (2.3)***	***307.0 (17.0)***	***293.3 (4.5)***	***92.6 (6.1)***
Polypeptides**	Colistin		89.8 (3.9)	149.4 (8.3)	405.2 (6.3)	61.5 (4.1)
	Any polypeptides		***89.8 (3.9)***	***149.4 (8.3)***	***405.2 (6.3)***	***61.5 (4.1)***
Penicillins*	Ampicillin	4 (1.7)	29.4 (1.3)	114.3 (6.3)	24.6 (0.4)	77.2 (5.1)
	Amoxicillin#	129.8 54.7)	93.0 (4.1)	82.1 (4.6)		546.5 (36.0)
	Penicillin V	4 (1.7)				
	Any penicillins	***137.9 (58.0)***	***122.4 (5.3)***	***196.4 (10.9)***	***24.6 (0.4)***	***623.7 (41.1)***
Macrolides**	Tylosin		242.3 (10.6)	329.8 (18.3)	1423.5 (22.1)	458.5 (30.2)
	Spiramycin	0.4 (0.2)	4.4 (0.2)	2.2 (0.1)		
	Erythromycin		72.5 (3.2)			
	Any macrolides	0.4 (0.2)	***319.2 (14.0)***	***331.9 (18.4)***	***1423.5 (22.1)***	***458.5 (30.2)***
Lincosamides	Lincomycin	1.6 (0.7)	45.5 (2.0)	5.2 (0.3)		
	Any lincosamides	***1.6 (0.7)***	***45.5 (2.0)***	***5.2 (0.3)***		
Diaminopyrimidines	Trimethoprim		20.4 (0.9)	16.7 (0.9)	35.2 (0.5)	11.1 (0.7)
	Any diaminopyrimidines		***20.4 (0.9)***	***16.7 (0.9)***	***35.2 (0.5)***	***11.1 (0.7)***
1st &2nd Cephalosporins	Cefuroxime	28.3 13.1)				
	Cefotaxime#			13.6 (0.6)		
	Cefalexin	38.9 16.2)	46.6 (2.0)			
	Cefdinir	2.9 (1.2)				
	Any 1st &2nd cephalosporins	***70.1 (29.5)***	***46.6 (2.0)***	**13.6 (0.6)**		

3rd	Ceftiofur#			10.4 (0.6)		
Cephalosporins**	Cefixim	11.3 (4.7)				
	Cefpodoxime	2.3 (1.0)				
	Cefadroxil	2.7 (1.1)				
	Any 3rd cephalosporins	***16.3 (6.8)***		***10.4 (0.6)***		
Amphenicols	Thiamphenicol		324.3 (14.2)	135.1 (7.5)		6.9 (0.5)
	Florfenicol		0.9 (0)	7.3 (0.4)		154.4 (10.2)
	Any amphenicols		***325.3 (14.2)***	***142.4 (7.9)***		***161.2 (10.7)***
Aminoglycosides*	Streptomycin		41.6 (1.8)	10.4 (0.6)		
	Spectinomycin		95.0 (4.2)	10.4 (0.6)		
	Kanamycin		4.3 (0.2)	5.2 (0.3)		
	Gentamicin		49.7 (2.2)	119.9 (6.7)	1229.1 (19.1)	49.1 (3.2)
	Any aminoglycosides		***190.6 (8.3)***	***146.0 (8.1)***		***49.1 (3.2)***
Grand total		***237.4 (100)***	***2,288 (100)***	***1,803 (100)***	***6,436 (100)***	***1,516 (100)***

Estimation of AMU for the Mekong Delta region

Details of human and animal body weight, the calculation of the total bodymass, the estimation of AMU and total antimicrobial consumption Mekong Delta region in 2019 both in frequency and quantities are presented in **Supplementary Material 3 (Table 1 and 2)**. The estimates of annual AMU for each species (including humans) for the Mekong Delta is displayed in Figure 3. The intensity of AMU in each animal depended on metrics used. The intensity was highest in 'animal standing bodymass' metric and lowest in 'biomass' metric.

Figure 3. Two-dimensional diagrams representing total annual AMU in humans and animals in the Mekong Delta region of Vietnam using four different metrics: (A) Treatment intensity (expressed as No.DDD per 1,000 inhabitant/animal-days), (B) mg of AAI related to 'standing animal bodyweight'; (C) mg of AAI related to 'biomass'; (D) mg of AAI related to 'No. PCUs'). The height of bars represents the population denominator, the width of bars the intensity of AMU by species. Colour code: Green: humans; yellow: pigs; dark blue: chickens; light blue: ducks; red: Muscovy ducks; black "*" ('other' species, including cattle, buffalo, goat, sheep, geese and quails, for which no AMU data are available). Vertical error bars represent the range between extreme scenarios (±50%).



The estimated total annual amounts of antimicrobials used (the area of the bars in Figure 3) in each of species (including humans) in the Mekong Delta region are presented in **Supplementary Material 3 (Table 3)**. A total of 7,055 billion doses-kg or 466.8 tonnes of AAIs were used. Pigs were the target of the greatest amounts of AMU, both in terms of frequency (40.6%) and quantity (36.1%). Human consumed a total of 1,334 billion doses (18.9% of all species) and 122.2 (26.2%) tonnes of of antimicrobials. However, per kg of body weight, human consumed less than 5% of the total consumption of all species).

Discussion

Quantification of AMU both in human and animals through the establishment of surveillance systems has been set by international agencies as a priority activity in order to successfully tackle the global threat of AMR [6, 31]. Although AMU surveillance systems both for humans and animals have already been established in a number of developed countries, (notably in European Union countries), in LMICs they are only starting to emerge. Most of these surveillance systems are based on sales data, and is limited by the difficulty of assigning species to the different antimicrobials sold since often products have a multi-species purpose.

Our survey was restricted to small-scale farming units. Extrapolations of human antimicrobial consumption data from the survey to all human populations in the Mekong Delta may not be appropriate, even though that in the region over 50% raise animals. Furthermore, since our study is not based a truly random sample, these results need to be taken with caution.The cross-sectional survey conducted during a fixed period (July) may also limit the validity of our results. It is likely that seasonal effects may affect AMU, both in humans and animals. In the study area, there is an increase in animal disease during the rainy season (June-November), and probably increased AMU (author's unpublished information). The increased diseases and relationship with AMU need to be further investigated. In our study, humans used approximately 15.2 DDDs per 1,000 inhabitants per day (TI=0.0152). These estimates were considerable modest, and lower than a previous published estimation for Vietnam (~32 DDDs), or even the 2019 EU average (20.1 DDDs, with a country range of 9.7–34.0) [32]. However, our study excluded antimicrobials used in human health care settings and hospitals. A recent report in Thailand suggests that AMU

for humans in Thailand in 2018 was considerably higher (74.4 DDDs) [33]. Children and the elderly used more, as in other countries.

Our study reports for Mekong Delta region, overall greater quantities of use in animals (91.9 ADD_{kg} or 912.3 mg/kg biomass or 1,591 mg/PCU) compared with published data from Thailand (711 mg/PCU in 2018), from EU (105.6 mg/PCU)and global estimates (240.5mg/kg biomass). In terms of treatment intensity, chickens consumed ~193 ADD_{kg} per 1,000 chicken-day. These values are about 50% lower than those measured in a previous study based on a large sample (382 ADD_{kg} per 1,000 chicken-days) [10]. This is probably a reflection that the flock size in our study included small (i.e. backyard) flocks which is likely to use lower amount of antimicrobials. This is likely to be explained since flocks in that study were 'all-in-all-out' (i.e. single age) which are known to use lesser amonts of antimicrobials, probably due to better disease control [20]. The antimicrobial amounts used in Muscovy duck farms were considerably higher magnitude compared with all other species. However, these estimates, were based on a small sample (the data are based on 12 farms raising Muscovy ducks). Therefore, future study should estimate sample size for each animal species separately in order to reduce the standard error.

The study results highlighted that the intensity of AMU in each animal species highly depended on metrics used. Estimation AMU in different metrics allowed us to compare with current AMU reported in other region. Besides that, due to these different, the interpretation of human AMU when compared with animal AMU need to take into account chosen metrics. The intensity was highest in 'animal standing bodymass' metric, lower in 'PCU' metric and lowest in 'biomass' metric. We found a greater diversity of antimicrobials used in animals (30 AAIs, belonging to 11 classes) compared with humans

(14 AAIs, belonging to 6 classes). The types of antimicrobial classes found in animals in this study were very similar to previous longitudinal study in the same area [10]. It is of concern that a high proportion of critical important classes (CIAs) were used in the community, especially some of the last resort antimicrobial for hospital-acquired infection. AAIs regarded of critical importance by WHO in animals (71.8%) were considerably higher magnitude compared with humans (47.4%), although the CIAs classes were different among human and animals species (penicillins and 3rd gen. cephalosporins in humans and tetracylines, penicillins and polypeptides in animals).

Using a relatively simple cross-sectional survey design, we measured AMU in different animal species, and compared it to that of humans in rural community settings using different metrics. We interviewed participants in their homes, allowing us to examine the antimicrobial products as well as their prescriptions and drug containers. This approach is remarkably affordable and appropriate to the modest economies of many LMICs for estimating total AMU in specific areas. On average, the on-farm interview in the cross-sectional study took approximately one hour (time require for completing the questionnaire and reviewing antimicrobial products used). In the longitudinal study, AMU data only collected during whole production cycle of a chicken flock. There were four visits per flock. In my experience, the person-time required per visit in a cross-sectional study is similar to that of a longitudinal study design. Therefore, the costs of the longitudinal-study are about four times higher. The cost to conduct a visit was between $15 and $20 in the study. We propose to expand this methodology in terms of geography (i.e. additional provinces), including a true representative sample of the study units to obtain a more precise and valid national AMU estimates.

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Conflict of interest statement

The authors declare no conflict of interest.

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Chapter 8

General discussion and conclusions

8.1 Contribution of this thesis

In this thesis I characterised AMU (what types of antimicrobials, in what quantities, when) in small-scale commercial chicken farming systems in the Mekong Delta region of Vietnam. The thesis also compared AMU in humans and as well as other animal species raised in farming communities in the Mekong Delta using different metrics. I also provided empirical evidence of the impact of untargeted use of antimicrobials on prevention and disease outcome. This understanding is crucial for designing effective intervention strategies to curb excessive antimicrobial consumption in low-resource settings.

8.2 Longitudinal versus cross-sectional studies

Antimicrobial consumption data may be gathered from farms either through longitudinal or cross-sectional surveys. Compared with unannounced 'one-off' visits to farms typical of cross-sectional studies, longitudinal study designs yield more accurate data on AMU coupled with other production variables over time. However, such studies are costly and time-consuming. In small-scale production units, farmers often do not keep records, and therefore this requires extra efforts by field teams in training farmers on data collection. Results from Chapter 4 indicated that overwhelmingly farmers used more antimicrobials during the 'brooding' period. Results from Chapter 7 demonstrated that quantitative AMU data can be gathered from a simple cross-sectional survey particularly if farmers are asked over AMU in their flocks/herds over a short period of time (7 days). This method is appropriate and affordable for a large scale survey (i.e. national survey) where longitudinal study might not be feasible.

8.3 Metrics of AMU

In all cases, estimates of AMU obtained from surveillance or farm studies are very sensitive to the chosen metric. Due to the different on the chosen weight of animal as well as animal population (census or production data) in each metric, comparison among different metrics need to be consider with caution. As shown in Chapter 7 AMU, the amounts appear largest when the weight of antimicrobial is related to the 'standing population', followed by 'PCU' and lowest in 'biomass' metrics (the latter two being related to production statistics). Similarly, results from Chapter 4 indicated that AMU amount are two folds higher when antimicrobial quantities are related to weight measured at treatment time' compared to slaughter time, when the animal is at its highest possible weight. A further consideration is that relating AMU to production output (i.e. kg of slaughtered animals) will, in situations of high mortality (such as the one described in this thesis) (Cuong, Phu et al. 2019; Carrique-Mas, Van et al. 2019), result in reduced estimates of AMU compared with situations of low mortality or no mortality at all.

This shows the challenge for establishing a 'single global metric' for AMU, and contributes to the difficulty in drawing comparisons across studies that may encompass different countries or production types. A recent study that reviewed 38 farm-based AMU surveillance systems from 16 countries confirmed that metrics used by these systems differed in many ways. The chosen indicators were dependent on many factors including the type of data collected, the type of analyses conducted and their respective output (Sanders, Vanderhaeghen et al. 2020). The current metrics adopted by ESVAC and OIE in their reports are 'weight-based' (mg/PCU and mg/kg of biomass) (EMA 2020; OIE 2020). These metrics are probably a good start, since may allow comparison in AMU across countries. However, these metrics do not reflect antimicrobials treatment intensity in different species, which might be important in the fight against

AMR. In its latest report (EMA 2020), EMA has established standardised units of measurement for reporting AMU in particular animal species (the 'defined daily dose' and 'defined course dose'). It is expected that this unit will be used in the future report of ESVAC alongside the current mg/PCU metric.

8.4 AMU in small-scale chicken production in the Vietnamese context

This thesis provides a full picture on AMU, including water and feed (323.4 mg and 84.8 mg per kg of chicken at slaughter time) in small-scale native chicken production in the Mekong Delta region of Vietnam. Over the life of chicken, in-feed AMU represents approximately 20% of total AMU. AMU consumed by chicken through water in this study (791mg/kg at treatment time) are much higher than global estimates in 2017 (68mg/PCU) (Tiseo, Huber et al. 2020). In term of treatment intensity, chicken in our study (results from Chapter 4) consumed three times more than global average levels (381 vs 138 doses per 1,000 chicken-days) (Cuong, Padungtod et al. 2018).

Results from Chapter 4 highlight the huge diversity of AAIs with a high proportion of CIA used in small-scale chicken farms. Results from Chapter 7 showed that consumption of CIAs in animals (71.8%) was of considerably higher magnitude than in humans (47.4%), although the CIAs classes were different among human and animals species (penicillins and 3rd gen. cephalosporins in humans and tetracylines, penicillins and polypeptides in animals). The high prevalence of colistin use and resistance found in the area (Nguyen, Phuong Yen et al. 2020) is of particular concern, since this is one of the antimicrobials of last resort for hospital-acquired infections in humans (Kadar, Kocsis et al. 2013).

8.5 Impact of AMU on animal and human health

As expected, given the high amounts of AMU in chicken production in the area, studies have identified a high prevalence of AMR in commensal flora microorganisms (i.e.

Escherichia coli) (Nguyen, Nguyen et al. 2016; Nhung, Cuong et al. 2015) and in food-borne pathogens (i.e. non-typhoidal *Salmonella* and *Campylobacter* spp.) (Carrique-Mas, Bryant et al. 2014; Tu, Hoang et al. 2015). A further concern is that antimicrobials products may be administerd towards the end of the production cycle (with or withdrawal tiem period), posing a risk of accumulation of antimicrobial residues in poultry meat (Patel, Marmulak et al. 2018). A number of recent studies have indicated a considerably high prevalence of antimicrobials residues in pork (23%) and in chicken meat samples (8.4%) (Ngoc Do, Yamaguchi et al. 2016; Nhung, Van et al. 2018).

8.6 Drivers of AMU in chicken production

The reasons that explain the observed high amounts of AMU are complex. One of the most important drivers is the high incidence of disease and mortality in this typical type of chicken prodution. A study demonstrated that AMU was most common during the 'brooding period' (3-4 first weeks of chicken life) when highest prevalence of disease was found (Carrique-Mas, Van et al. 2019). Furthermore, due to the complexity of infectious disease etiology in the area (Van, Yen et al. 2019), there is evidences that the probability of the effective treatment often very low (approximately 57%) (Choisy, Van Cuong et al. 2019). Farmers, when facing the failure of an antimicrobial treatment course, might look for new treatment courses with new antimicrobial classes, resulted in even higher amount of AMU overall. It is particularly worrying if this phenomenon is common in reality, since antimicrobials can be easily purchased over the counter at extremely low retail prices (Dung, Truong et al. 2020). Farmers' behavior on disease management practices might explain such high quantities of AMU. A study showed that farmers believed the cost of AMU cheaper than other disease control management practices; and even these costs are more expensive, they would lead to more effective disease prevention (Truong, Doan et al. 2019). It is believed that authorities veterinarian were the most trusted sources of advice on AMU and many of them also own a private veterinary drug shops which were the main sources of supply antimicrobial products in the region. To increase profitability, these private drug shops might sell antimicrobial products even if it is not necessary. A study has demonstrated that high level of AMU are associated with high density of the veterinary pharmacies (Phu, Giao et al. 2019).

In Vietnam, the legislation states that antimicrobial products intended for human usage can only be purchased with a medical prescription (MOH). Compliance with this regulation is monitored by the inspection services of the Health Ministry and the local

Health Services. However, it is unclear how the sales of antimicrobials is managed since there is no compulsory recording system or database in place (only for addictive drugs, poisonous medicament, psychotropic medicines and pre-substances used as drugs). In veterinary medicine, none of these regulations existed at present.

Result from Chapter 6 indicated that antimicrobial products aiming for prophylactic purpose were used in half of the time of total AMU. This is because high ratio (65%) of antimicrobial products contain the ambiguous labelling by providing guidelines for both therapeutic and prophylactic AMU (Yen, Phu et al. 2019). This type of labels encouraged the prophylactically usage in healthy chicken that resulted in high amount AMU overall.

8.7 Quality of antimicrobial products

Vietnam is one of the largest markets of pharmaceutical industry in Southeast Asia. In the region, many poor quality pharmaceutical products are also manufactured (Kelesidis and Falagas, 2015). Vietnam only started to implement Good Manufacturing Practices (GMP) in 2004, and is lagging behind other countries in SEA region. Vietnamese authorities now require that all manufacturers of human medicinal products or vaccines must comply with GMP standards (GMP-ASEAN, GMP-WHO or an equivalent from 2012 (Joint Circular No.01/2012/TTLT-BYT-BTC) and in veterinary medicine from 2016 (Decree No. 35/2016/ND-CP). The country currently has more than 170 GMP human (78 veterinary) pharmaceutical manufacturing facilities, more than 9,000 pharmacies, 10,000 veterinary drug stores and more than 40,000 human licensed products (including over 12,000 veterinary products) (DAV 2020 and DAH 2020). Due to limited financial and human resources, the number of products that routinely investigated is very low.

The most common type of poor quality antimicrobial products fall into three categories: poor workmanship (i.e. antimicrobial does not meet advertised quality), counterfeit (i.e. does not contain genuine active ingredients) and degraded drugs (Le Minor, 2011). Although a number of low quality antimicrobial products were reported in Vietnam in the past (WHO 1999), the number of counterfeit and substandard antimicrobial drugs in human medicine in Vietnam is unknown. However, a recent study showed that Vietnam had the largest proportion (among six countries of the study) of non-licensed antimicrobials products (high possibility of low quality products) circulating in the market. This situation is particularly more worrying since self-medication with antimicrobial products are wide-spread in Vietnam (55·2% of antimicrobial products dispensed without prescription) (Nga and Huong, 2021).

In veterinary medicine, a recent study showed that about 7% of antimicrobial products (n=144) contained less than half of the labelled content concentrations (Huong and Thuy, 2021). Other study highlighted that only about 30% of veterinary antimicrobial products contained all AAIs within 10% of the declared strength (Yen and Phu, 2019). The usage of low quality antimicrobial drugs can lead to treatment failures in both animal and human health. Substandard antimicrobial drugs may resulted in the increase of morbidity, mortality and antimicrobial resistance. The low quality of antimicrobial products highlight the need of consistent and sustainable monitoring system for both human and veterinary medicines.

8.8 A ban of AMU for prophylactic purposes (including AGPs)

Result from Chapter 6 indicated that antimicrobial products aiming for prophylactic purpose were used in half of the time of total AMU. A number of other studies showed that routine preventive AMU is still common in chicken production systems (Carrique-Mas, Trung et al. 2014; Coyne, Arief et al. 2019). Results from this study model demonstrated that there were no impact of prophylactic AMU on the prevention of diseases in chicken. A number of recent studies have been highlighted the overall poor effects of AGPs in poultry productivity (Hamid, Zhao et al. 2019; Kumar, Chen et al. 2018). In contrast, positive effects are only observed in low biosecurity production systems (Laxminarayan 2015). However, this small marginal productivity gains often offset by the high mortality rates found in this region.

Vietnam is currently engaged in legislative efforts leading to progressive reductions of antimicrobials use in animals. A recent Decree issued by the VN Government (13/2020/ND-CP) includes the timeframe for a ban of AMU for prophylactic purposes (including AGPs), with phased bans for different antimicrobials classes. Data from this thesis showed that prophylactic AMU in chicken production in Vietnam is of considerable greater impact than AGPs, since the in-feed AMU represents a small fraction of total AMU and the most common AAIs found in chicken feeds often have little impact on human health.

8.9 Limitations of this thesis

This study focused on local breed chicken farms with average flocks size from 100 to 1,500 birds (data for chapters 4, 5, 6). A large proportion of farms with larger size (industrial farms) were excluded. This might limit the representativeness of the AMU estimates. Although, only small fraction (26.1%) corresponded to chickens raised in

industrial systems in Vietnam, it is forecasted that the major increase of AMU mostly driven by increased number of the intensification of farming systems in the near future.

It is not possible to define prophylactic and therapeutic AMU when both AMU and clinical signs were reported in the same week. Although, the approach used in Chapter 6 provided a clear definition of prophylactic and therapeutic of AMU, still, about 50% of the data were excluded, thus decreased the statistical power of this study.

8.10 Conclusions and recommendations

Through the quantification of AMU in animals and in humans in small-scale farms in Mekong Delta region of Vietnam and the analysis of AMU in relation to disease of chicken flocks, this research yielded a number of important results.

First, results from both longitudinal and cross-sectional studies showed significant discrepancies between AMU measurement metrics. The ‘dose-based’ and ‘weight-based’ metrics resulted in quantitative different estimates in levels of AMU. It is importance to take into account when comparing AMU data across studies.

Second, a complete picture of antimicrobials administered (both through water and feed) to chicken flocks in the Mekong Delta region of Vietnam, showed a high fraction of AMU in chicken production consists of CIAs. In addition, in terms of dosing, chickens are predominantly medicated during the ‘brooding period’.

Third, compared with antimicrobials administered to chicken flocks through water, consumption of AGPs in feed represents a relatively small fraction of total AMU. Furthermore, a considerable number of feed formulations did not comply with Government regulations.

Fourth, study models clearly indicated the ineffective impact of prophylactic AMU and palliative effect of therapeutic AMU. Prophylactic AMU should not be used if unnecessary since it does not reduce the probability of disease. Therapeutic AMU should be used with a proper diagnostic.

Finally, using a One Health approach, this study demonstrated that AMU can be estimated from a relatively simple cross-sectional survey, although results are very sensitive to the chosen metric. This methodology can be used in AMU surveillance in low-resource settings, allowing to focus reduction efforts AMU in particular animal species.

Based on the results of this research and current situation in Vietnam, the following recommendations are put forward for the next steps in monitoring and reducing the AMU in Vietnam as well as in other similar LMICs:

- The methodology used in Chapter 7 should be applied for a national survey of AMU because of its appropriate and affordable (cost-effective) approach in the Vietnamese context. This method should be further expanded in terms of geography (i.e. additional Vietnamese provinces) as well as performing a true representative sampling of the study units (i.e. random sampling).

- The establishment of surveillance systems for AMU is imperative in Vietnam (as well as in other LMICs). Surveillance systems should allow establishing comparisons of AMU in different production types and geographical locations, and can be used to monitor changes in AMU over time. Both ‘dose-based’ and ‘weight-based’ metrics should be used in Vietnam since the Vietnamese government collects comprehensive statistical data on animal production. The

collection of ‘dose-based’ data is relatively simple (i.e. number of days using antimicrobials), although it cannot be expected to be fully accurate since many farmers actually overdose (and some underdose).

- Authorities should implement long-term, sustainable training programs targeting different stakeholders (farmers, veterinarian, veterinary pharmacists, feed producers etc.) to increase their awareness of the problem of AMR and the importance of AMU reduction. Such training should emphasize the need to improve day-old chick quality and farming practices (i.e. biosecurity, cleaning and disinfection, brooding management, vaccination). A feasible approach is to deliver these training programs through the veterinary authority (Sub-Department of Animal Health in each province). A recent study showed that reducing current high levels of AMU through the provision of veterinary advice (i.e. improve diagnostic capacity) resulted in considerable reductions in AMU and mortality (Phu, Cuong et al. 2021). Since most veterinary antimicrobials are procured in local vet drug shops, the role of the veterinary pharmacists as gatekeepers of AMU should be reinforced.

- Efforts to reduce AMU should focus the farming sector since quantitatively more than 80% amounts of AMU corresponds to animal production. Although new legislations on AMU management has been released in Vietnam. A number of specific policies should be implemented. For example, the quality of day-old chicks are often not reliable and there are no law related to the quality control management on the day-old chicks. Other issues might relate to the ambigious labelling of antimicrobial products, espcially for prophylactic usage

purpose since it encouraged the unneccessary AMU. All antimicrobial products should indicate for specific usage, not for multiple purposes.

- The policies on antimicrobial pricing should be revised to discourage the use of CIAs. Currently antimicrobials intended for animals are disproportionally cheap. The use of a number of antimicrobial alternative products already available in the market (i.e. plant essential oils, yeast extracts, probiotic, prebiotic etc.) should be promoted.

- The last recommendation is that the human and animal AMU as well as AMR data should be collected in parallel. Due to complexity of the AMR phenomenon, complementation of these data might provide the best possible solution to combat AMR in both animals and humans..

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Supplementary Materials Chapter 5

Antimicrobial use through consumption of medicated feeds in chicken flocks in the Mekong Delta of Vietnam: a three-year study before a ban on antimicrobial growth promoters

Supplementary Material S1. List of products containing AAIs and its active ingredients concentration

No	Product code	Stage of production	No of AAIs	Formulation	AAIs concentration (mg/kg of feed)								Description	No. of farm	No. of flock
					Avilamycin	Bacitracin	Chlortetracycline	Colistin	Enramycin	Flavomycin	Oxytetracyline	Virginimycin			
1	FE001	Broodin g	1	Crumb					10				Certain	42	127
2	FE002	Growing	1	Pellet					10				Certain	9	11
3	FE004	Growing	1	Pellet					10				Certain	37	109
4	FE005	Broodin g	1	Crumb			50						Certain	6	13
5	FE006	Growing	1	Pellet			50						Certain	4	10
6	FE007	Broodin g	1	Pellet			50						Certain	8	19
7	FE011	Broodin g	1	Crumb			50						Certain	4	6
8	FE013	Growing	1	Pellet			10-50						Certain	1	2
9	FE015*	Broodin g	1	Crumb			50	50*			50		Ambiguous	3	4
10	FE017	Finishin g	1	Pellet		50							Certain	1	1
11	FE019	Broodin g	1	Crumb		30							Certain	27	79

12	FE023	Brooding	1	Crumb				10			Certain	2	2
13	FE024	Brooding	1	Crumb			50				Certain	13	26
14	FE025	Growing	1	Pellet			50				Certain	9	24
15	FE026	Finishing	1	Pellet	10	50		10	10		Ambiguous	3	5
16	FE033	Brooding	1	Crumb	10	50		10	10		Ambiguous	2	2
17	FE036	Growing	1	Pellet				1-10			Certain	5	5
18	FE037	Brooding	1	Pellet			50				Certain	7	16
19	FE040	Brooding	1	Mask		50					Certain	1	3
20	FE042	Brooding	1	Pellet			50				Certain	1	1
21	FE043	Growing	1	Pellet			50				Certain	1	1
22	FE045	Finishing	1	Pellet	10	50		10	10		Ambiguous	1	1
23	FE047	Brooding	1	Crumb		50		10			Ambiguous	3	3
24	FE048	Growing	1	Pellet				10			Certain	3	3
25	FE053	Brooding	1	Pellet		50		10			Ambiguous	2	5
26	FE055*	Finishing	1	Pellet		50		15*		5	Ambiguous	1	4
27	FE058	Brooding	1	Pellet		4-50		1-10			Ambiguous	2	2

28	FE059	Broodin g	1	Crumb							5-15	Certain	6	6
29	FE066*	Growing	1	Pellet	15	125*			10	2		Ambiguous	2	2
30	FE067	Broodin g	1	Crumb			50					Certain	1	2
31	FE074*	Broodin g	1	Crumb	15	125*			10	2		Ambiguous	4	4
32	FE080	Broodin g	1	Crumb					1-10			Certain	1	2
33	FE090*	Finishin g	1	Pellet		50-100*		60-160*				Ambiguous	2	2
34	FE094*	Broodin g	1	Crumb		50-100*		60-160*				Ambiguous	1	1
35	FE104*	Broodin g	2	Crumb			75-150*	100-200*				Certain	1	1

*Products that have AAIs concentration higher than permitted Vietnamese regulation

Supplementary Materials Chapter 7

Feasibility study of a field survey to measure antimicrobial usage in humans and animals in the Mekong Delta region of Vietnam

Nghiên cứu về kiến thức và thực hành trong việc sử dụng thuốc và thuốc kháng (trụ) sinh trong gia đình và chăn nuôi tại Đồng Tháp, Việt Nam

Đơn vị Nghiên cứu Lâm sàng Đại học Oxford

Bệnh viện Bệnh Nhiệt đới, TP Hồ Chí Minh, Việt Nam

1	Ngày phỏng vấn (ngày/tháng/năm)	
2	Người được phỏng vấn	
3	Số điện thoại	
4	Người thực hiện phỏng vấn (họ và tên)	
5	Ấp	
6	Xã	
7	Huyện	
8	Mã trại/hộ gia đình	
9	Toạ độ GPS	

Nghiên cứu của chúng tôi nhằm tìm hiểu về kiến thức và thực hành trong việc sử dụng thuốc và thuốc kháng sinh trong gia đình và chăn nuôi tại các hộ chăn nuôi nhỏ ở tỉnh Đồng Tháp, thông qua việc phỏng vấn trực tiếp người nông dân. Anh/chị đã được phổ biến về nội dung của nghiên cứu và đồng ý tham gia.

Anh/chị đồng ý bắt đầu phỏng vấn chứ?

A. THÔNG TIN CHĂN NUÔI VÀ SỬ DỤNG THUỐC VÀ THUỐC KHÁNG SINH TRONG CHĂN NUÔI

A1. THÔNG TIN CHUNG		
Các loài gia súc đang chăn nuôi (chọn tất cả các đáp án đúng)	**Sử dụng thuốc trong tuần vừa qua?**	
☐ Gà	☐ Có	☐ Không/Không nhớ
☐ Vịt	☐ Có	☐ Không/Không nhớ
☐ Vịt xiêm	☐ Có	☐ Không/Không nhớ
☐ Ngỗng	☐ Có	☐ Không/Không nhớ
☐ Chim cút	☐ Có	☐ Không/Không nhớ
☐ Heo	☐ Có	☐ Không/Không nhớ
☐ Bò	☐ Có	☐ Không/Không nhớ
☐ Trâu	☐ Có	☐ Không/Không nhớ
☐ Dê, cừu	☐ Có	☐ Không/Không nhớ
☐ Cá	☐ Có	☐ Không/Không nhớ
☐ Tôm	☐ Có	☐ Không/Không nhớ
☐ Khác (ghi rõ)	☐ Có	☐ Không/Không nhớ

A2. THÔNG TIN CHĂN NUÔI VÀ VIỆC SỬ DỤNG THUỐC VÀ THUỐC KHÁNG SINH TRONG CHĂN NUÔI					
A2A. Tên loài 1:					
A2B. Độ tuổi (theo tháng)		**1** [__ __]	**2** [__ __]	**3** [__ __]	**4** [__ __]
A2C. Số con		__ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __
A2D. Mục đích chăn nuôi (thịt, trứng, giống v.v…, theo từng nhóm tuổi)					
A2E. Số con dùng thuốc (trên tổng cộng)		__ __ __ __ /__ __ __ __	__ __ __ __ /__ __ __ __	__ __ __ __ /__ __ __ __	__ __ __ __ /__ __ __ __
A2F. Bao bì/đơn thuốc vẫn còn lưu giữ?	Có	☐	☐	☐	☐
	Không	☐	☐	☐	☐
A2G. Thông tin dùng theo nhóm tuổi (chỉ điền nếu xác định hoặc nghi ngờ là kháng sinh)		**1**	**2**	**3**	**4**
Sản phẩm thuốc 1	**Tên sản phẩm**				
	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
Sản phẩm thuốc 2	**Tên sản phẩm**				

	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
Sản phẩm thuốc 3	**Tên sản phẩm**				
	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
Sản phẩm thuốc 4	**Tên sản phẩm**				
	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
A2H. Lời khuyên của ai trong việc dùng thuốc?	Người bán thuốc	☐	☐	☐	☐
	Bác sĩ/nhân viên thú y	☐	☐	☐	☐
	Bạn bè, người thân	☐	☐	☐	☐
	Kinh nghiệm cá nhân	☐	☐	☐	☐
	Khác (ghi rõ)				

A2. THÔNG TIN CHĂN NUÔI VÀ VIỆC SỬ DỤNG THUỐC VÀ THUỐC KHÁNG SINH TRONG CHĂN NUÔI					
A2A. Tên loài 2:					
A2B. Độ tuổi (theo tháng)		**1** [__ __]	**2** [__ __]	**3** [__ __]	**4** [__ __]
A2C. Số con		__ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __
A2D. Mục đích chăn nuôi (thịt, trứng, giống v.v…, theo từng nhóm tuổi)					
A2E. Số con dùng thuốc (trên tổng cộng)		__ __ __ __ /__ __ __ __	__ __ __ __ /__ __ __ __	__ __ __ __ /__ __ __ __	__ __ __ __ /__ __ __ __
A2F. Bao bì/đơn thuốc vẫn còn lưu giữ?	Có	☐	☐	☐	☐
	Không	☐	☐	☐	☐
A2G. Thông tin dùng theo nhóm tuổi (chỉ điền nếu xác định /nghi ngờ là kháng sinh)		**1**	**2**	**3**	**4**
Sản phẩm thuốc 1	**Tên sản phẩm**				
	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
Sản phẩm thuốc 2	**Tên sản phẩm**				

	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
Sản phẩm thuốc 3	**Tên sản phẩm**				
	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
Sản phẩm thuốc 4	**Tên sản phẩm**				
	Thời gian dùng (trên 7 ngày)	**__ __/07**	**__ __/07**	**__ __/07**	**__ __/07**
	Lý do				
A2H. Lời khuyên của ai trong việc dùng thuốc?	Người bán thuốc	☐	☐	☐	☐
	Bác sĩ/nhân viên thú y	☐	☐	☐	☐
	Bạn bè, người thân	☐	☐	☐	☐
	Kinh nghiệm cá nhân	☐	☐	☐	☐
	Khác (ghi rõ)				

B. KIẾN THỨC

B1. Kiến thức về kháng sinh trên người:

Lưu ý:

- Khoanh tròn một lựa chọn
- Không tiết lộ người dân trả lời đúng hay sai trong quá trình hỏi. Có thể giải thích sau khi kết thúc tuỳ thời gian cho phép.
- Khi người dân không hiểu câu hỏi, thay đổi cách diễn đạt. Nếu vẫn không biết hoặc không hiểu, khoanh tròn Không biết/không hiểu câu hỏi (99).
- Ghi chép lại tất cả các câu trả lời đáng chú ý (e.g. người dân không biết vi khuẩn/vi-rút nhưng hiểu kháng sinh chống nhiễm trùng vết thương, khái niệm lờn thuốc v.v…)

B1A. Trước khi tham gia vào nghiên cứu này, anh/chị đã nghe tới khái niệm thuốc kháng sinh/thuốc trụ sinh?

1. Có => mời tham gia trò chơi, sau đó hỏi tiếp bảng câu hỏi từ A2 đến A11
2. Không => chuyển sang phần B

B1B. Thuốc kháng sinh được dùng để chữa **cảm lạnh** hoặc **cảm cúm**.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

B1C. Thuốc kháng sinh được dùng để **trị** bệnh **sốt siêu vi, sốt xuất huyết**.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

B1D. Nếu đang sử dụng một liều thuốc kháng sinh, **khi nào có thể dừng**?

1. Khi cảm thấy khoẻ hơn
2. Khi đã uống hết thuốc theo chỉ dẫn hoặc theo đơn
99. Không biết/không hiểu câu hỏi

B1E. Thuốc kháng sinh **đã sử dụng** có thể được **dùng tiếp cho bạn bè/người thân**, nếu họ mắc những triệu chứng hoặc bệnh tương tự.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

B1F. Khi bị bệnh cần sử dụng thuốc kháng sinh, có thể **mua tiếp** loại kháng sinh đó nếu lần sau mắc phải bệnh hoặc triệu chứng tương tự.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

B2. Kiến thức về kháng sinh trong chăn nuôi:

B2A. Thuốc kháng sinh được dùng để **trị** bệnh **cúm gia cầm, viêm gan vịt**.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

B2B. Thuốc kháng sinh được dùng để **trị** bệnh ***E. coli*** trên gà, vịt.

2. Sai
99. Không biết/không hiểu câu hỏi

B2C. Thuốc kháng sinh được dùng để **thúc đẩy tăng trưởng** gia súc và gia cầm.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

B2D. Thuốc kháng sinh được sử dụng chữa bệnh **phổ biến trên người** hơn là trên gia súc và gia cầm.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

B2E. Tồn dư kháng sinh trong thịt có thể bị **hấp thụ** bởi con người.

1. Đúng
2. Sai
99. Không biết/không hiểu câu hỏi

C. THÔNG TIN GIA ĐÌNH VÀ VIỆC SỬ DỤNG THUỐC VÀ THUỐC KHÁNG SINH TRONG GIA ĐÌNH								
C1A. Số thành viên đã ký phiếu đồng ý tham gia:_____ **Số trẻ em dưới 18 tuổi được bố/mẹ thay mặt ký**:_____								
C1B. Tên thành viên (khoanh tròn thành viên trả lời)			**1** _____	**2** _____	**3** _____	**4** _____	**5** _____	**6** _____
C1C. Tuổi (theo năm, viết 01 nếu nhỏ hơn 1 tuổi)			[__ __]	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]
C1D. Giới tính	Nam		☐	☐	☐	☐	☐	☐
	Nữ		☐	☐	☐	☐	☐	☐
C1E. Trình độ **học vấn**	Không đi học		☐	☐	☐	☐	☐	☐
	Cấp 1		☐	☐	☐	☐	☐	☐
	Cấp 2		☐	☐	☐	☐	☐	☐
	Cấp 3		☐	☐	☐	☐	☐	☐
	Cao đẳng/ nghề		☐	☐	☐	☐	☐	☐
	Đại học trở lên		☐	☐	☐	☐	☐	☐
C1F. Nghề nghiệp và **thu nhập** hàng năm	Làm nông	Lương thực	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]
		Cây trái/hoa màu	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]

(chọn tất cả các đáp án đúng)	Chăn nuôi	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]
	Buôn bán nhỏ	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]
	Lao động	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]
	Cơ quan nhà nước	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]
	Công ty/doanh nghiệp tư nhân	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]	☐ [_ _ _,_ _ _, _ _ _]
	Khác (ghi rõ)	_____ [_ _ _,_ _ _, _ _ _]	_____ [_ _ _,_ _ _, _ _ _]	_____ [_ _ _,_ _ _, _ _ _]	_____ [_ _ _,_ _ _, _ _ _]	_____ [_ _ _,_ _ _, _ _ _]	_____ [_ _ _,_ _ _, _ _ _]
C1G. Tiếp xúc với gia súc/gia cầm trong gia đình?	Hàng ngày	☐	☐	☐	☐	☐	☐
	Hơn 1 lần/tuần	☐	☐	☐	☐	☐	☐
	Hơn 1 lần/ tháng	☐	☐	☐	☐	☐	☐
	Hơn 1 lần/ 6 tháng	☐	☐	☐	☐	☐	☐
	Không tiếp xúc	☐	☐	☐	☐	☐	☐

C1H. Sử dụng **thuốc** trong vòng **3 tháng qua?**	**Có** **Số lần dùng,** nếu nhớ	☐ [__ __]	☐ [__ __]	☐ [__ __]	☐ [__ __]	☐ [__ __]	☐ [__ __]
	Không **- Lần cuối,** nếu nhớ**?**	☐ _____	☐ _____	☐ _____	☐ _____	☐ _____	☐ _____
	Không nhớ **- Lần cuối**, nếu nhớ?	☐ _____	☐ _____	☐ _____	☐ _____	☐ _____	☐ _____
C1I. Bao bì/đơn thuốc vẫn còn lưu giữ?	**Có**	☐	☐	☐	☐	☐	☐
	Không Có nhớ tên thuốc? (đánh dấu vào ô nếu có)	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
C1J. Thông tin thuốc (chỉ điền nếu xác định hoặc nghi ngờ là kháng sinh)		**Thành viên 1**	**Thành viên 2**	**Thành viên 3**	**Thành viên 4**	**Thành viên 5**	**Thành viên 6**
Sản phẩm thuốc 1	**Tên sản phẩm**						
	Số lần dùng (trên tổng số lần dùng thuốc)	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __
	Thời gian dùng (theo ngày)	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]

	Lý do						
Sản phẩm thuốc 2	**Tên sản phẩm**						
	Số lần dùng (trên tổng số lần dùng thuốc)	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __
	Thời gian dùng (theo ngày)	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]
	Lý do						
Sản phẩm thuốc 3	**Tên sản phẩm**						
	Số lần dùng (trên tổng số lần dùng thuốc)	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __
	Thời gian dùng (theo ngày)	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]
	Lý do						

Sản phẩm thuốc 4	**Tên sản phẩm**						
	Số lần dùng (trên tổng số lần dùng thuốc)	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __	__ __ /__ __
	Thời gian dùng (theo ngày)	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]	[__ __]
	Lý do	_____	_____	_____	_____	_____	_____
C1K . Lời khuyên của ai trong việc dùng thuốc?	Người bán thuốc	☐	☐	☐	☐	☐	☐
	Bác sĩ/nhân viên y tế	☐	☐	☐	☐	☐	☐
	Bạn bè, người thân	☐	☐	☐	☐	☐	☐
	Kinh nghiệm cá nhân	☐	☐	☐	☐	☐	☐
	Khác (ghi rõ)	☐	☐	☐	☐	☐	☐

Ghi chú:

Appendix 2.

Table 1. The ‘standing bodymass’ of people living in the Mekong Delta region of Vietnam. It was estimated using age-gender-weight metrics from census (1) and published data (2).

Age (years)	Total population (1)			Average weight (kg) (2)		Estimated bodymass (kg)		
	Males	Females	Total	Males	Females	Males	Females	Total
0 to 4	779,689	677,961	1,457,650	8.0	7.8	6,237,511	5,288,093	11,525,605
5 to 9	743,489	646,657	1,390,146	14.7	14.7	10,929,295	9,505,858	20,435,153
10 to 14	684,944	603,585	1,288,529	25.6	27	17,534,568	16,296,790	33,831,357
15 to 19	625,648	570,054	1,195,702	41.4	35.4	25,901,830	20,179,916	46,081,745
20 to 40	3,029,801	2,852,915	5,882,716	58.4	50.8	176,940,398	144,928,062	321,868,460
41 to 65	2,611,166	2,637,830	5,248,996	58.4	50.8	152,492,079	134,001,773	286,493,852
> 65	535,755	805,206	1,340,961	58.4	50.8	31,288,117	40,904,446	72,192,563
Total	9,010,493	8,794,207	17,804,700	-	-	421,323,798	371,104,937	792,428,735

(1). *Population pyramid of Vietnam, 2019*. 2019; Available from: https://www.populationpyramid.net/viet-nam/2019/.

(2) Carrique-Mas, J.J., et al., *An estimation of total antimicrobial usage in humans and animals in Vietnam.* Antimicrob Resist Infect Control, 2020. **9**: p. 16.

Table 2. The estimation of animal ‘standing bodymass’, animal ‘biomass’ and animal ‘PCU’. It was estimated from number of animal (from census (1), production data (2)) and weight of animals (from mid-point weight (3), slaughter weight (4) and treatment weight (5). Mid-point weight of meat animal equals 50% slaughter weight.

Species	Production type	No. animals		Weight of animals			Total animal weight		
		No. animals at a given time (census data) (1)	No. slaughtered animals (production data) (2)	Mid-point weight (kg) (3)	Slaughter weight (kg) (4)	Treatment weight (kg) (5)	Standing bodymass (kg) (1)*(3)	Biomass (kg) (2*4)	PCU (kg) (2*5)
Pig	Meat	1,487,452	2,974,904*	39.3	78.6	65.0	58,456,864	233,827,454	193,368,760
	Breeder	198,481	198,481	240.0	240.0	240.0	47,635,440	47,635,440	47,635,440
Chicken	Meat	40,853,000	74,381,000	0.9	1.8	1.0	36,767,700	133,885,800	74,381,000
	Breeder	12,003,000	12,003,000	1.8	1.8	1.0	21,605,400	21,605,400	12,003,000
Duck	Meat	15,923,000	46,070,000	1.0	2.0	1.1	15,923,000	92,140,000	50,677,000
	Breeder	11,388,000	11,388,000	2.0	2.0	1.1	22,776,000	22,776,000	12,526,800
Muscovy duck	Meat	1,959,000	3,760,000	1.6	3.2	1.7	3,134,400	12,032,000	6,392,000
	Breeder	222,000	222,000	3.2	3.2	1.7	710,400	710,400	377,400
Quails	Meat	2,871,900	6,980,000	0.07	0.13	0.08	186,674	907,400	558,400
	Breeder	319,100	319,100	0.13	0.13	0.08	41,483	41,483	25,528
Cattle	Meat	808,955	292,386	75.0	150.0	140.0	60,671,625	43,857,900	40,934,040
	Breeder	70,707	70,707	300.0	300.0	425.0	21,212,100	21,212,100	30,050,475
Buffalo	Meat	20,736	7,053	150.0	300.0	140.0	3,110,400	2,115,900	987,420
	Breeder	2,303	2,303	500.0	500.0	425.0	1,151,500	1,151,500	978,775
Goat	Meat	358,592	250,724	37.5	75.0	20.0	13,447,200	18,804,300	5,014,480
	Breeder	39,843	39,843	75.0	75.0	75.0	2,988,225	2,988,225	2,988,225
Sheep	Meat	838	560	37.5	75.0	20.0	31,425	42,000	11,200
	Breeder	93	93	75.0	75.0	75.0	6,975	6,975	6,975
Geese	Meat	203,000	164,000	1.6	3.2	1.7	324,800	524,800	280,440
	Breeder	55,000	55,000	3.2	3.2	1.7	176,000	176,000	93,500

(1),(2) Thống kê chăn nuôi Việt Nam 01/01/2020. Available at: https://channuoivietnam.com/thong-ke-chan-nuoi/

(4) OIE Annual Report on Antimicrobial Agents Intended for Use in Animals: Methods Used. Available at: https://www.frontiersin.org/files/Articles/462898/fvets-06-00317-HTML/image_m/fvets-06-00317-t002.jpg

(5) European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) Sales Data and Animal Population Data Collection Protocol. Available at: https://www.ema.europa.eu/en/documents/other/european-surveillance-veterinary-antimicrobial-consumption-esvac-web-based-sales-animal-population_en.pdf

*Pig production data were estimated from census data.

Supplementary Material 1**.**

Table 1. Socio-demographic characteristics of 316 residents of 101 small-scale farming households.

	Interviewee N=101 (%)	Other participants N=215 (%)	All participants N=316 (%)
District			
Cao Lanh	21 (20.8)	50 (23.3)	71 (22.5)
Chau Thanh	20 (19.8)	45 (20.9)	65 (20.6)
Lai Vung	21 (20.8)	39 (18.1)	60 (19)
Thap Muoi	20 (19.8)	53 (24.7)	73 (23.1)
Tam Nong	19 (18.8)	28 (13)	47 (14.9)
Age			
<5	0 (0)	31 (14.4)	31 (9.8)
5-19	0 (0)	65 (30.2)	65 (20.6)
20-40	27 (26.7)	43 (20)	70 (22.2)
41-65	56 (55.4)	48 (22.3)	104 (32.9)
>65	18 (17.8)	28 (13)	46 (14.6)
Gender			
Male	83 (82.2)	81 (37.7)	164 (51.9)
Female	18 (17.8)	134 (62.3)	152 (48.1)
Frequency of contact with animals			
No contact	2 (2)	112 (52.0)	114 (36.0)
Daily	96 (95)	77 (35.8)	173 (54.7)
Weekly	1 (1)	12 (5.6)	13 (4.1)
More often	2 (2)	14 (6.5)	16 (5.0)
Education achivement*			
No school	5 (5)	20 (9.3)	25 (7.9)
Primary school	41 (40.6)	48 (22.3)	89 (28.2)
Secondary school	33 (32.7)	36 (16.7)	69 (21.8)
High school or higher	22 (21.8)	15 (7)	37 (11.7)

*Applicable to residents aged >18 years.

Table 2. Species and production types of animals raised in 101 small-scale farming households. NC: Not calculated. NA: Not available.

Species	Farming type	No. farms (N=101) (%)	Age (weeks) (median) [IQR]	Flock size (median) [IQR]
Chicken		72 (71.3)	12 [4-24]	30 [15-70]
	Meat	53 (52.5)	10.5 [4-14]	40 [20-200]
	Fighting	22 (21.8)	12 [4-27]	30 [20-50]
	Breeding/layer	21 (20.7)	48 [30-66]	10 [6-23]
Duck		55 (54.5)	8 [4-25]	100 [40-700]
	Meat	37 (36.6)	6 [4-9]	80 [37-182]
	Breeding/layer	21 (20.8)	28 [24-46]	1,500 [500-2,300]
Pig		20 (19.8)	15 [8-51]	10 [3-12]
	Meat	17 (16.8)	12 [8-20]	10 [7-12]
	Breeding	6 (5.9)	48 [32-96]	3 [2-10]
Muscovy duck		12 (11.9)	4 [2-12]	30 [10-77]
	Meat	11 (10.9)	4 [2-8]	40 [13-100]
	Breeding/layer	3 (3)	52 [32-81]	4 [3-11]
Fish		11 (10.9)	14 [12-19]	1,000 [162-1,000]
	Meat	9 (8.9)	14 [12-17]	1,000 [120-1,000]
	Breeding	2 (2)	19 [13-25]	NA
Cattle		6 (5.9)	80 [52-240]	2 [1-2]
	Meat	5 (5.0)	56 [40-92]	2 [1-2]
	Breeding	2 (2.0)	276 [258-294]	1 [NC]
Frog		4 (4.0)	5 [3-8]	15,000 [15000-55000]
	Meat	4 (4.0)	4 [3-5]	22,500 [15,000-67,500]
	Breeding	1 (1.0)	32 [32-32]	500 [NC]
Goat		2 (2.0)	32 [26-40]	5 [NC]
	Meat	1 (1.0)	20 [20-20]	5 [NC]
	Breeding	1 (2.0)	40 [36-44]	5 [NC]
Geese	*Breeding/layer*	2 (2.0)	67 [48-85]	2 [NC]

Supplementary Material S2. Animal and human ADDs/DDDs

Product	Species where used	No. AAI	Administration route	AAI_1	ADDkg_1	AAI_2	ADD2kg_2
AB030	pig	2	injection	oxytetracycline	3.8	thiamphenicol	7.5
AB032	pig	2	injection	florfenicol	7.5	tylosin	21.4
AB009	pig	1	injection	tylosin	0.9		
AB071	pig	1	injection	enrofloxacin	3.8		
AB015	pig	2	oral	colistin	3.0	gentamicin	5.0
AB016	pig	2	oral	ampicillin	9.4	colistin	4.5
AB036	pig	1	oral	amoxicillin	10.5		
AB073	pig	2	oral	amoxicillin	20.3	gentamicin	6.1
AB074	pig	2	oral	sulfamethoxazole	40.5	trimethoprim	8.1
AB012	poultry	1	injection	doxycycline	8.4		
AB013	poultry	1	injection	marbofloxacin	3.8		
AB014	poultry	2	injection	tylosin	7.5	thiamphenicol	30.0
AB017	poultry	1	injection	ceftiofur	3.8		
AB023	poultry	1	injection	cefotaxime	9.8		
AB024	poultry	2	injection	spectinomycin	15.0	lincomycin	7.5
AB028	poultry	1	injection	florfenicol	1.8		
AB030	poultry	2	injection	oxytetracycline	7.5	thiamphenicol	15.0
AB043	poultry	1	injection	marbofloxacin	7.5		
AB044	poultry	1	injection	amoxicillin	0.8		
AB045	poultry	2	injection	oxytetracycline	3.8	thiamphenicol	7.5
AB045	poultry	2	injection	lincomycin	3.8	spectinomycin	7.5
AB047	poultry	2	injection	oxytetracycline	7.5	thiamphenicol	15.0
AB052	poultry	1	injection	spiramycin	3.0		
AB055	poultry	2	injection	tylosin	11.3	spectinomycin	7.5
AB056	poultry	1	injection	oxytetracycline	30.0		
AB057	poultry	2	injection	lincomycin	4.2	spectinomycin	11.3
AB001	poultry	2	oral	doxycycline	13.2	ampicillin	18.9
AB002	poultry	1	oral	enrofloxacin	11.8		
AB003	poultry	1	oral	enrofloxacin	18.8		
AB004	poultry	1	oral	sulfamethoxazole	18.7		
AB005	poultry	1	oral	sulfaquinoxaline	8.4		
AB006	poultry	1	oral	doxycycline	16.9		
AB007	poultry	2	oral	streptomycin	7.5	oxytetracycline	15.0
AB008	poultry	1	oral	oxytetracycline	1.5		
AB010	poultry	2	oral	gentamicin	75.0	doxycycline	131.3
AB011	poultry	1	oral	enrofloxacin	8.4		
AB016	poultry	2	oral	colistin	4.5	ampicillin	9.4
AB018	poultry	1	oral	florfenicol	10.5	doxycycline	5.3
AB019	poultry	2	oral	colistin	0.5	ampicillin	3.2
AB020	poultry	2	oral	ampicillin	7.5	colistin	1.1
AB021	poultry	2	oral	colistin	3.5	trimethoprim	5.1
AB022	poultry	1	oral	kanamycin	12.4		
AB025	poultry	1	oral	sulfadimidine	33.8		
AB026	poultry	2	oral	florfenicol	0.8	doxycycline	8.4
AB027	poultry	2	oral	tylosin	8.4	doxycycline	16.9

AB029	poultry	2	oral	tylosin	7.5	sulfadimethoxine	15.0
AB031	poultry	2	oral	oxytetracycline	7.5	colistin	0.3
AB033	poultry	1	oral	tylosin	4.5		
AB034	poultry	1	oral	thiamphenicol	24.8		
AB035	poultry	2	oral	spiramycin	0.5	trimethoprim	1.5
AB037	poultry	1	oral	enrofloxacin	10.5		
AB038	poultry	2	oral	gentamicin	3.0	tylosin	12.0
AB039	poultry	1	oral	norfloxacin	15.0		
AB040	poultry	1	oral	oxytetracycline	1.0		
AB041	poultry	2	oral	tetracycline	18.8	tylosin	7.5
AB042	poultry	1	oral	oxytetracycline	18.8		
AB046	poultry	2	oral	amoxicillin	15.0	erythromycin	15.0
AB048	poultry	2	oral	cefalexin	7.5	gentamicin	7.5
AB049	poultry	2	oral	sulfadimidine	93.2	sulfaquinoxaline	28.4
AB050	poultry	1	oral	oxytetracycline	2.5		
AB051	poultry	2	oral	tylosin	31.5	colistin	9.4
AB053	poultry	2	oral	doxycycline	7.5	tylosin	3.8
AB054	poultry	1	oral	streptomycin	12.4		
AB058	poultry	2	oral	gentamicin	7.5	doxycycline	13.1
AB059	poultry	2	oral	amoxicillin	16.9	colistin	3.4
AB060	poultry	1	oral	enrofloxacin	8.4		
AB061	poultry	2	oral	ampicillin	1.9	kamycin	3.8
AB062	poultry	1	oral	colistin	1.7		
AB063	poultry	2	oral	colistin	4.5	oxytetracycline	5.6
AB063	poultry	2	oral	colistin	4.5	tetracycline	5.6
AB064	poultry	2	oral	tylosin	5.0	tetracycline	12.4
AB065	poultry	1	oral	norfloxacin	2.3		
AB066	poultry	1	oral	oxytetracycline	5.1		
AB067	poultry	1	oral	enrofloxacin	8.4		
AB068	poultry	1	oral	amoxicillin	9.4		
AB069	poultry	2	oral	lincomycin	0.8	spectinomycin	1.9
AB070	poultry	2	oral	trimethoprim	4.5	colistin	3.6
AB072	poultry	2	oral	colistin	1.7	oxytetracycline	5.5
H1	human	1	oral	ciprofloxacin	18.5		
H2	human	1	oral	cefuroxime	27.7		
H3	human	1	oral	cefdinir	13.8		
H4	human	1	oral	amoxicillin	32.3		
H5	human	1	oral	cefuroxime	9.2		
H6	human	1	oral	penicillin v	23.1		
H7	human	1	oral	spiramycin	9.2		
H8	human	1	oral	ampicillin	23.1		
H9	human	1	oral	tetracycline	23.1		
H10	human	1	oral	amoxicillin	36.9		
H11	human	1	oral	amoxicillin	17.7		
H12	human	1	oral	cefuroxime	18.5		
H13	human	1	oral	cefalexin	18.5		
H14	human	1	oral	cefalexin	9.2		
H15	human	1	oral	amoxicillin	9.2		
H16	human	1	oral	cefalexin	8.1		

H17	human	1	oral	spiramycin	5.8
H18	human	1	oral	cefalexin	18.5
H19	human	1	oral	amoxicillin	18.5
H20	human	1	oral	cefuroxime	18.5
H21	human	1	oral	cefpodoxime	1.8
H22	human	1	oral	cefadroxil	9.2
H23	human	1	oral	amoxicillin	18.5
H24	human	1	oral	cefpodoxime	4.6
H25	human	1	oral	amoxicillin	23.1
H26	human	1	oral	amoxicillin	23.1
H27	human	1	oral	ofloxacin	7.4
H28	human	1	oral	cefuroxime	11.5
H29	human	1	oral	amoxicillin	23.1
H30	human	1	oral	cefixim	9.2
H31	human	1	oral	amoxicillin	40.4
H32	human	1	oral	lincomycin	23.1

Supplementary material 3.

Table 1. Calculations of antimicrobial consumption for humans in the Mekong Delta region of Vietnam based on data from the survey of 101 farms.

		Age group							Total
		0 to 4	5 to 9	10 to 14	15 to 19	20 to 40	41 to 65	> 65	
Survey	Standing bodymass (kg)	376.4	582.9	859.4	551.8	3844.8	5686	2519.2	
	mg AAI/kg stading bodymass	966.6	230.9	147.9	0	96	165.3	232.8	237.3
	No. DDD_{kg}/kg stading bodymass	10.5	8.9	3.9	0	2.8	4.8	9.8	5.9
	Treatment intensity	0.0289	0.0246	0.0108	0	0.007	0.0134	0.0269	0.0152
Mekong Delta	Standing bodymass (kg)	11,525,605	20,435,153	33,831,357	46,081,745	321,868,460	286,493,852	72,192,563	792,428,735
	Bodymass-days (kg-days)	4,206,845,825	7,458,830,845	12,348,445,305	16,819,836,925	117,481,987,900	104,570,255,980	26,350,285,495	289,236,488,275
	No. DDD_{kg}	121,018,853	181,872,862	131,942,292	0	901,231,688	1,375,170,490	707,487,117	3,418,723,302
	mg AAI	11,140,649,793	4,718,476,828	5,003,657,700	0	30,899,372,160	47,357,433,736	16,806,428,666	115,926,018,883
	mg AAI/kg stading bodymass								146.2 [73.1-219.4]
	No. DDD_{kg}/kg stading bodymass								4.3 [2.1-6.4]
	Treatment intensity								0.0118 [0.0059-0.0177]

Table 2. Calculation of antimicrobial usage of animals raised in the Mekong Delta region of Vietnam.

Species	Sub-species	Data from the survey		Data from Appendix 2	Numerator		Denominator (Data from Appendix 2)				Estimated AMU Mekong Delta region [50% lower-upper estimated AMU level] (% of total)			
		Frequency (No. DDDkg/kg) (1)	Quantity (mg/kg) (2)	Weight of standing animals (kg) (3)	No. of doses (No.DDDkg) (4) = (1)*(3)	Quantity (mg) (5)=(2)*(3)	Bodymass-days (kg-days) (6)	Weight of standing animals (kg) (7)	Biomass (kg) (8)	PCU (kg) (9)	Treatment intensity (TI) No.DDD per 1,000 animal-days Σ(4)/Σ(6)	mg/kg standing animals Σ(5)/Σ(7)	mg/kg biomass Σ(5)/Σ(8)	mg/kg PCU Σ(5)/Σ(9)
Pig	Meat	87.1	1287.4	58,456,864	1,858,431,379,139	75,257,366,199	21,336,755,214	58,456,864	233,827,454	193,368,760				
	Breeder	57.9	1954	47,635,440	1,006,703,571,240	93,079,649,760	17,386,935,600	47,635,440	47,635,440	47,635,440				
	All	73.2	1,516.6	106,092,304	2,865,134,950,379	168,337,015,959	38,723,690,814	106,092,304	281,462,894	241,004,200	200.5 [100.2-300.8] (20.1)	1,586.7 [793.1-2,380.0] (14.0)	598.0 [299.0-897..1] (15.7)	698.4 [349.2-1,047.7] (10.7)
Chicken	Meat	81.8	1711.3	36,767,700	1,097,773,218,900	62,920,565,010	13,420,210,500	36,767,700	133,885,800	74,381,000				
	Breeder	43.8	787.8	21,605,400	345,405,529,800	17,020,734,120	7,885,971,000	21,605,400	21,605,400	12,003,000				
	All	70.3	2,288.3	58,373,100	1,443,178,748,700	79,941,299,130	21,306,181,500	58,373,100	155,491,200	86,384,000	192.6 [96.3-288.9] (19.3)	1,369.4 [684.7-2,054.2] (12.1)	514.1 [257.0-771.1] (13.5)	925.4 [462.7-1,388.1] (14.2)
Duck	Meat	80	2224.4	15,923,000	464,951,600,000	35,419,121,200	5,811,895,000	15,923,000	92,140,000	50,677,000				
	Breeder	90.3	1227.9	22,776,000	750,685,572,000	27,966,650,400	8,313,240,000	22,776,000	22,776,000	12,526,800				
	All	80.3	1,803.2	38,699,000	1,215,637,172,000	63,385,771,600	14,125,135,000	38,699,000	114,916,000	63,203,800	220.0 [110.0-330.0] (22.0)	1,637.9 [881.9-2,465.8] (14.5)	551.5 [275.7-827.3] (14.5)	1,002.8 [501.4-1,504.3] (15.4)
Muscovy duck	Meat	163.8	7923.6	3,134,400	187,396,372,800	24,835,731,840	1,144,056,000	3,134,400	12,032,000	6,392,000				
	Breeder	34.7	651.7	710,400	8,997,571,200	462,967,680	259,296,000	710,400	710,400	377,400				
	All	136.3	6,436.6	3,844,800	196,393,944,000	32,970,313,440	1,403,352,000	3,844,800	12,742,400	6,769,400	373.4 [186.7-560.1] (37.4)	6,579.9 [3,289.9-9,869.9] (58.1)	1,985.3 [992.6-2,978.0] (52.2)	3,737.2 [1,868.6-5,5605.8] (57.3)
Human		5.9	237.3		3,656,823,550	122,229,500,679	289,236,488,275	792,428,735	792,428,735	792,428,735	11.8 [5.9-17.7] (1.3)	154.2 [77.1-231.3] (1.4)	154.2 [77.1-231.3] (4.1)	154.2 [77.1-231.3] (2.)
Total											998.5 [499.5-1498.6] (100)	11,328.1 [5,664.0-16,992.1] (100)	3,803.1 [1,901.5-5,704,6] (100)	6,518 [3,259-9,777] (100)

Table 3. Percentage of total usage across species (including human)

Species	No. of doses (No.DDDkg) (%)		Quantity (mg) (%)	
Pig	2,865,134,950,379	(40.6)	168,337,015,959	(36.1)
Chicken	1,443,178,748,700	(20.5)	79,941,299,130	(17.1)
Duck	1,215,637,172,000	(17.2)	63,385,771,600	(13.6)
Muscovy duck	196,393,944,000	(2.8)	32,970,313,440	(7.1)
Human	1,334,740,595,750	(18.9)	122,229,500,679	(26.2)
Total	7,055,085,410,829	(100)	466,863,900,808	(100)

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Assessment of Drivers of Antimicrobial Usage in Poultry Farms in the Mekong Delta of Vietnam: A Combined Participatory Epidemiology and Q-Sorting Approach

Dinh Bao Truong[1,2†], Hoang Phu Doan[2,3†], Vinh Khanh Doan Tran[2], Van Cuong Nguyen[1], Tuan Kiet Bach[4], Chalalai Rueanghiran[5], Aurélie Binot[6], Flavie L. Goutard[3,6], Guy Thwaites[1,7], Juan Carrique-Mas[1,7] and Jonathan Rushton[8]*

[1] Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam, [2] Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh, Vietnam, [3] Faculty Veterinary Medicine, Kasetsart University, Bangkok, Thailand, [4] Sub Department of Animal Health and Production, Cao Lãnh, Vietnam, [5] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, [6] ASTRE, CIRAD, INRA, University Montpellier, Montpellier, France, [7] Centre for Tropical Medicine and Global Health, Oxford University, Oxford, United Kingdom, [8] Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom

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Edited by:
Ioannis Magouras,
City University of Hong Kong,
Hong Kong

Reviewed by:
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Tariku Jibat Beyene,
Kansas State University, United States

***Correspondence:**
Dinh Bao Truong
dinhbao.truong@hcmuaf.edu.vn

† These authors have contributed equally to this work

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In the Mekong Delta of Vietnam, poultry farmers use high amounts of antimicrobials, but little is known about the drivers that influence this usage. We aimed to identify these drivers using a novel approach that combined participatory epidemiology (PE) and Q-sorting (a methodology that allows the analysis of the subjectivity of individuals facing a common phenomenon). A total of 26 semi-structured collective interviews were conducted with 125 farmers representative of the most common farming systems in the area (chickens, meat ducks, and mobile grazing ducks), as well as with 73 farmers' advisors [veterinarians, veterinary drug shop owners, and government veterinarians/commune animal health workers (CAHWs)] in five districts of Dong Thap province (Mekong Delta). Through these interviews, 46 statements related to the antimicrobials' perceived reliability, costs, and impact on flock health were created. These statements were then investigated on 54 individuals (28 farmers and 26 farmers' advisors) using Q-sorting interviews. Farmers generally indicated a higher propensity for antimicrobial usage (AMU) should their flocks encounter bacterial infections (75.0–78.6%) compared with viral infections (8.3–66.7%). The most trusted sources of advice to farmers were, in decreasing order: government veterinarian/CAHWs, their own knowledge/experience, veterinary drug shop owners, and sales persons from pharmaceutical and feed companies. The highest peak of AMU took place in the early phase of the production cycle. Farmers and their advisors showed considerable heterogeneity of attitudes with regards to AMU, with, respectively, four and three discourses representing their views on AMU. Overall, farmers regarded the cost of

AMU cheaper than other disease management practices implemented on their farms. However, they also believed that even though these measures were more expensive, they would also lead to more effective disease prevention. A key recommendation from this finding would be for the veterinary authorities to implement long-term sustainable training programs aiming at reducing farmers' reliance on antimicrobials.

Keywords: antimicrobial usage, Q-sorting, participatory epidemiology, farmers' attitude, discourse

INTRODUCTION

The misuse (over- and under-use) of antimicrobials in animal production is one of the contributing factors of the global emergency of antimicrobial resistance (AMR) (1). Levels of antimicrobial usage (AMU) in low- and middle-income countries (LMICs) are particularly high (2), and are expected to increase markedly over coming years due to intensification of animal production and increased demand for animal protein (3, 4). In the Mekong Delta of Vietnam farmers typically use large amounts of antimicrobials to raise poultry, and a high incidence of disease has been reported in chicken flocks (5). A recent study showed that, on average, 470 mg antimicrobial compounds were used to produce one meat chicken, and most of the AMU was aimed at preventing, rather than treating disease (6, 7). A survey conducted in Cambodia on small-scale pig farms showed that the farmer's own judgment was the most important determinant associated with AMU (8). Another survey on small- and medium-scale pig farms in northeastern Thailand indicated that two thirds (68%) of small-scale farmers decided themselves whether or not to give antimicrobials to their animals, whereas all medium-scale farmers discussed antimicrobial treatments with a veterinarian (9). When using antimicrobials to treat disease, European pig farmers were more interested in the short-term impact on their herds' health than in the AMR "side effects" (10). A study on Vietnamese poultry farms confirmed that, from the farmers' point of view, the main target is to maintain the highest possible number of birds alive until end of production (11). A study of poultry farmers in the Mekong Delta found that the farmers' sources of advice were: drug sellers (56%), followed by the district veterinarian (18%), and farmers colleagues (12%) (6). However, there is a gap in knowledge on the farmers' perception of the antimicrobials' effectiveness and the socio-economic factors driving AMU in the Mekong Delta of Vietnam. This knowledge is critical for the design and implementation of intervention strategies.

The study used two well-documented methods to fill this knowledge gap: Participatory Epidemiology and Q-sorting. Participatory epidemiology (PE) is the systematic use of participatory approaches and methods to improve the understanding of diseases and options for animal disease control. PE involves communities to define and prioritize animal health problems, and to improve veterinary service delivery, control and/or surveillance of diseases (12). PE draws on widely accepted techniques of participatory rural appraisal, ethno-veterinary surveys, and qualitative epidemiology (13). Q-sorting is a qualitative method used to analyse the subjective perception of individuals in relation to a particular situation or phenomenon. Q-sorting helps identify trends and convergences of opinions (14), and has been used in a wide variety of research areas, such as political subjectivity (14), public health (15, 16), veterinary science (17), and rural sociology (18, 19).

Specific objectives of the study were: (a) to identify the relative frequency of disease in flocks and the farmers' propensity for using antimicrobials should disease appear; (b) to identify the timing of antimicrobial administration in relation to the amounts used; (c) to define the sources of advice and procurement of antimicrobials to farmers; (d) to identify farmers' positive and negative opinions on AMU; and (e) to investigate socio-economic factors influencing farmers' attitudes on AMU.

Abbreviations: AMU, Antimicrobial usage; PE, Participatory epidemiology; PCA, Principal Component Analysis.

MATERIALS AND METHODS

Study Population

The study was conducted in Dong Thap province (Mekong Delta of Vietnam), from December 2017 to March 2018. The Mekong Delta is a relatively homogeneous agro-ecological region, and Dong Thap province is representative of this region. We chose the five (of 12) districts with the highest poultry populations, and focused on the three main types of poultry production in this area. The production cycle was typically 4 months for meat chickens, 2–3 months for meat ducks, and 2–3 years for free-ranging ducks. The study population consisted of (a) farmers, including owners of chicken, meat duck and free-grazing duck flocks, and (b) farmer' advisors, comprising veterinary drug shop owners, CAHWs and government veterinarian.

Farmers and veterinary drug shop owners were randomly selected from the official census held at the sub-Department of Animal Health and Production in Dong Thap (SDAH-DT). Government veterinarian/CAHWs were also randomly selected from the staff list. We aimed to select 250 participants of the five types of stakeholders (50 per district), organized into 25 semi-structured collective interviews (CIs) (five per district). The term "CI" was chosen over "FGI" (focus group interview), since the group of participants was heterogeneous and we were more seeking for a consensus in the answers, rather than exploring controversial points of view. The latter is normally applicable to FGI. Each CI session included 10 participants of one type of stakeholder. The number of CI chosen for each type of stakeholder (five) was based on (a) the concept of "saturation point," that estimates that 90% themes within a research topic

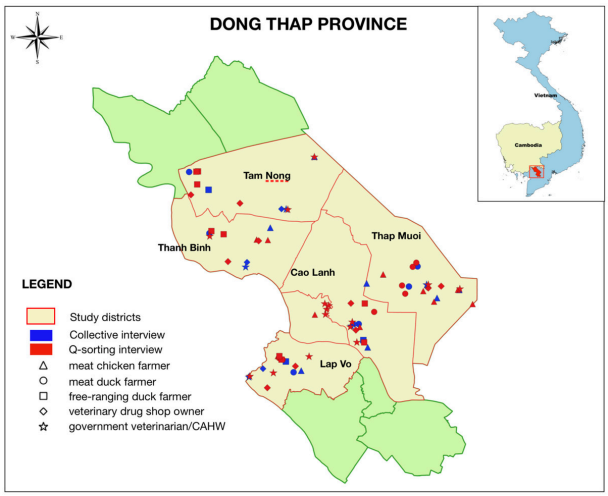

FIGURE 1 | Map of study areas in Dong Thap province. Blue: geographical location of CIs; red: geographical location of participants of Q-sorting interviews; triangle: chicken farmers; circle: meat duck farmers; square: free-grazing duck farmers; rhombus: veterinary drug shop owners; star: government veterinarian/CAHWs.

are normally discoverable by conducting three to six group interviews with each type of stakeholder (20); and (b) the objective of capturing the diversity opinion of farmers who raised different types of poultry and lived in different districts within the province. For Q-sorting, 55 participants of CIs were randomly selected and were invited to participate in the Q-sorting phase by conducting individual interviews. This number of participants was based on the sampling criteria described by Brown (14). The selection of participants formed a heterogeneous group based on type of production, gender, age, education level, location and experience in raising poultry (farmers). In addition, five government veterinarians were invited to take part in the Q-sorting step, since they are thought to play a very important role in Vietnamese animal production. All five interviewers and facilitators had previously been trained in PE and Q-sorting methodologies. All steps were conducted in Vietnamese since over 95% of the population in this province are ethnic Vietnamese. The interview sessions (CI or Q-sorting interview) took about 1 h each. Data were collected during the discussions with a digital voice recorder, and during the PE exercises information was recorded using written notes and pictures. All participants were initially contacted by staff affiliated to the SDAH-DT. For each interview (CI or Q-sorting interview), written informed consent was obtained from all participants before enrolment. The location of the interview sessions is shown in **Figure 1**.

The study was performed in several sequential steps (**Supplementary Figure 1**) (17).

Collection of Descriptive Data Using PE Tools

Qualitative and semi-quantitative data about existing opinions about AMU were collected during CI with farmers and their advisors using both open-ended questions and a checklist organized in four thematic areas (**Supplementary 1**): (a) Characterize diseases in poultry farms (chicken and duck farmers separately), and describe farmers' strategies to prevent and control them; (b) Identify the timing of AMU (chicken and duck farmers separately); (c) Identify sources of advice and procurement of antimicrobials to farmers (farmers and their advisors); and (d) Identify positive and negative opinions on AMU (farmers and their advisors). Various participatory tools were used to collect the data. These included pair-wise ranking for (a), time line tool for (b), proportional piling for (b) and (c), and flow-chart for (d). The PE data collection was performed following published guidelines (21). At the end of the discussions on each thematic area, a consensus was sought. The facilitators summarized the main CI findings and asked: "*Do you all agree, or would you like to change something*?" Minority opinions were discussed in all cases, after which the group was asked to accept or reject those opinions.

Q-Sorting Interviews and In-Depth Post Q-Sorting Interviews

The raw data gathered in the PE phase were used to generate a list of statements. This process included: screening, summarizing the data, creating statements, and modifying statements in line with research team's opinions. The Q-sorting interview process has been described by Truong et al. (17). In short, participants were invited to read, score, and allocate statements into a quasi-normal grid of 46 boxes according to their option. Statements were scored from −3 (extremely disagree) to +3 (extremely agree) (i.e., seven discrete options). After Q-sorting interview, additional questions were asked to participants to clarify the reasons behind their choice of extreme values for statements.

Statistical Analyses

The non-standardized data (semi-quantitative) collected from the pair-wise ranking exercise were transformed and standardized with a rank-score process (21). CI participants were asked to list poultry diseases important in their area and rank them according to their importance. This rank was transformed into a score, and results were averaged across CI for each disease using the median and inter-quartile range. CI participants were also asked whether they would use antimicrobials should they encounter each of the diseases listed. The information generated was converted into a probability of AMU conditional to each disease listed being present, and binomial confidence intervals were calculated around these estimates. Other descriptive (semi-quantitative) data were summarized using median score (MS), interquartile range (for proportional piling exercise), and percentage (for frequency of information from the flow-chart exercise) where appropriate. Data from each Q-sorting interview were introduced into two correlation matrices (one for farmers and one for farmers' advisor group) that included statements as observations and participants as variables (22, 23) (**Supplementary Figure 2**). Principal component analysis (PCA) was performed on these correlation matrices in order to shortlist a number of factors (3–10) for the next step of analysis (17, 24). The number of factors selected was based on

the level of heterogeneity of participants' views, subjectively evaluated by the researchers (25). The correlation matrices generated were subjected to factor analysis separately in order to identify discourses that best characterized clusters of participants (25, 26) as described by Truong et al. (17). Rotation of *k* factors (chosen from 3 to 10) was carried out during the factor analysis on the basis of (a) the best factor combination could explain 40% of cumulative percentage of variation; and (b) each factor comprised at least 5% of the total Q sort that loaded distinctly and significantly (14, 17, 27). Respondents who were assigned to more than one factor were considered as confounders. The respective score of each statement were recalculated through factor analysis process and it represented the relative score of one statement given by one particular discourse. The outcome was *k* discourses which were represented by *k* selected factors at the beginning. These discourses were a hypothetical Q-sorting that had been reconstructed from the factor scores (17, 25) (**Supplementary 2**, **Supplementary Table 2**). Statements were regarded as consensus points when the difference between the scores attained in any pair of factors were not statistically significant (based on the standard error of differences) (27). Transcripts from CIs and Q-sorting interviews were stored and extracted using the "rqda" package in R (28). Those data were not being analyzed statistically but were integrated in discussion section as explanation for the results obtained from exercise in the field. All data analyses were performed using R statistical software (29).

RESULTS

Study Population

A total 26 CIs with 198 participants were conducted: five CIs with veterinary drug shop owners (34 participants), five CIs with government veterinarians/CAHWs (39 participants), seven CIs with chicken farmers (49 participants), six CIs with meat duck farmers (30 participants), and three CIs with free-ranging duck farmers (46 participants). The actual number of CIs and participants were slightly different from the planned number due to unpredictable field constrains. Of the 60 participants that had been invited in the Q-sorting interview, six were removed from the analysis either because of their misunderstanding of the Q-sorting instructions or unwillingness to complete the procedure. The analysis therefore included 28 farmers and 26 advisors. The demographic features of participants are shown in **Table 1**.

Descriptive Data

The CIs identified a total of 15 poultry infectious diseases (data not shown). Diseases were described using their local names (often designing the etiological agent). The three chicken diseases that ranked highest across all CIs were: Gumboro disease; mycoplasmosis; and Newcastle Disease (**Figure 2**). The duck diseases that ranked highest were duck hepatitis and duck plague (**Figure 3**). The CIs indicated that antimicrobial use if flocks were affected by bacterial disease was greatest for pasteurellosis (87.5%; i.e., 14 CIs would use antimicrobials among 16 CIs reporting this disease), colibacillosis (72.7%; 8/11), and mycoplasmosis (78.6%; 11/14). For viral diseases usage was related to: Highly Pathogenic Avian Influenza (HPAI) (40.0%; 4/10); ND (33.3%; 3/9); Gumboro disease (25.0%; 3/12); Duck plague (18.2%; 2/11); Duck hepatitis (8.3%; 1/12). Other causative agents and usage included hepatitis (66.7%; 2/3), coccidiosis (87.5%; 7/8), and aspergillosis (100.0%; 6/6). A total of 15.0% of CIs reported prophylactic antimicrobial use during seasonal transitions.

In quantitative terms, most of the antimicrobials were administered during the second month of the production cycle (MS 43.0 and 45.5% for chicken and duck production, respectively), followed by the first month of the production cycle (MS 19.0 and 29.0%) (**Figure 4**).

The most trusted sources of advice to farmers were government veterinarian/CAHWs (MS = 28.0), their own knowledge/experience (MS = 26.0), the veterinary drug shop owners (MS = 21.0), and sales persons from pharmaceutical and feed companies (MS = 0.0). The farmers' advisor group ranked the veterinary drug shop owner as the most important source of advice to farmers (MS = 29.5), followed by government veterinarians/CAHWs (MS = 22.5), the farmers' own knowledge/experience (MS = 19.5), sales persons of pharmaceutical companies (MS = 4.0) and sales persons of feed companies (MS = 3.5) (**Figure 5**). Five positive and seven negative outcomes of AMU were identified. Similarly, eight positive and five negative outcomes were identified because of not using antimicrobials, respectively (**Table 2**).

Q-Sorting Interviews

Based on the list of opinions from different stakeholders, 46 final statements were generated, representing the spectrum of opinions on AMU around four thematic areas: (a) Farmers' confidence in antimicrobials as a tool for prevention, treatment or growth promotion; (b) Antimicrobial administration logistics; (c) Costs of the antimicrobials used; and (d) Impact of AMU/AMR on animal health/productivity and human health (See list of the statements related to each of these areas in **Supplementary Table 1**).

PCA and Factor Analysis

Among the farmer group, four discourses (F1–F4) were identified. These explained 17, 15, 13, and 10% of the total variability (55% cumulative variance). Among farmers' advisors, three discourses (A1–A3) were identified, explaining 18, 17, and 15% of the total variability (50% cumulative variance). Six respondents were considered as confounders. The discourses were labeled based on the score attained on some relevant statements. The statement numbers followed by their respective scores are shown within brackets (i.e., 46, −2 means statement number is 46 and its score is −2). The summary of the reconstructed Q-sorting from a total of seven discourses in both groups was shown in **Figures 6**, **7**.

Discourse Description

Discourse F1 represented farmers who displayed knowledge of AMU in poultry production ("Awareness"). Farmers allocated to discourse F2 were reliant on antimicrobials to raise poultry ("Dependency"). Discourse F3 represented farmers who freely use antimicrobials without consulting anyone else ("Initiative"). Discourse F4 constituted a group of farmers who had limited

TABLE 1 | Demographic description of participants involved in CI and Q-sorting interviews phases of the study.

	Collective interviews participants					Q-sorting interviews participants				
	Total (*n* = 198)	**Chicken farmers (*n* = 49)**	**Meat duck farmers (*n* = 30)**	**Free-ranging ducks farmers (*n* = 46)**	**Farmers' advisors (*n* = 73)**	**Total (*n* = 54)**	**Chicken farmers (*n* = 11)**	**Meat duck farmers (*n* = 8)**	**Free-ranging duck farmers (*n* = 9)**	**Farmers' advisors (*n* = 26)**
Age in years [median [interquartile range]]	41.0 [34.0–50.0]	45.0 [35.0–54.0]	44.0 [37.0–51.5]	42.5 [37.0–47.8]	35.0 [33.0–43.0]	43.0 [34.3–51.0]	51.0 [48.5–62.0]	41.0 [32.0–51.8]	46.0 [42.0–53.0]	38.0 [33.5–43.0]
GENDER										
Male (%)	178 (89.9)	43 (87.8)	29 (96.7)	45 (97.8)	61 (83.6)	48 (88.9)	10 (90.9)	8 (100.0)	9 (100.0)	21 (80.8)
Female (%)	20 (10.1)	6 (12.2)	1 (3.3)	1 (2.2)	12 (16.4)	6 (11.1)	1 (9.1)	0 (0.0)	0 (0.0)	5 (19.2)
DISTRICT										
Cao Lanh (%)	43 (21.7)	10 (20.4)	7 (23.3)	9 (19.6)	17 (23.3)	15 (27.8)	2 (18.2)	2 (25.0)	2 (22.2)	9 (34.6)
Lap Vo (%)	43 (21.7)	7 (14.3)	9 (30.0)	11 (23.9)	16 (21.9)	10 (18.5)	1 (9.1)	2 (25.0	2 (22.2)	5 (19.2)
Tam Nong (%)	40 (20.2)	9 (18.4)	0 (0.0)	18 (39.1)	13 (17.8)	7 (13.0)	0 (0.0)	0 (0.0)	3 (33.3)	4 (15.4)
Thanh Binh (%)	25 (12.6)	5 (10.2)	0 (0.0)	8 (17.4)	12 (16.4)	8 (14.8)	2 (18.2)	0 (0.0)	2 (22.2)	4 (15.4)
Thap Muoi (%)	47 (23.7)	18 (36.7)	14 (46.7)	0 (0.0)	15 (20.5)	14 (25.9)	6 (54.5)	4 (50.0)	0 (0.0)	4 (15.4)

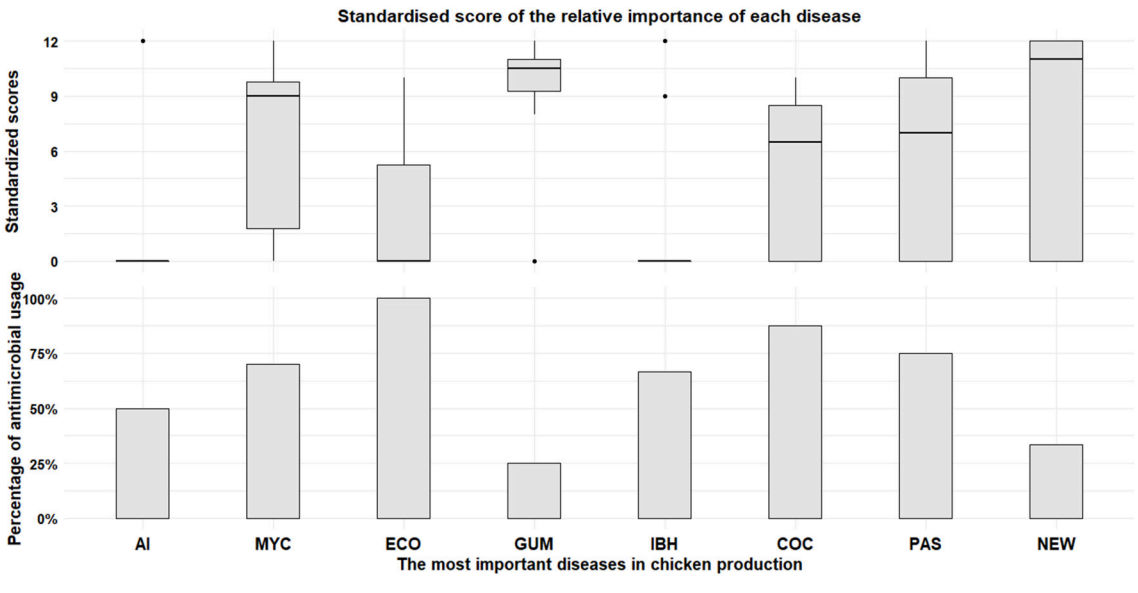

FIGURE 2 | Classification of the most important diseases (top) and frequency of AMU conditional to disease present in their farm (bottom) for chicken farms. AI, Highly Pathogenic Avian Influenza; MYC, Mycoplasmosis; ECO, *Escherichia coli*; GUM, Gumboro; IBH, Inclusion Body Hepatitis; COC, Coccidiosis; PAS, Pasteurellosis; NEW, Newcastle.

knowledge on AMU ("Imperfectness"). Advisors following discourse A1 ("Negativism") thought that farmers generally lack knowledge on AMU. Discourse A2 ("Hopefulness") was assigned to advisors who believed that farmers' knowledge and attitude although inadequate, will eventually improve. Finally, discourse A3 ("Advice dependency") characterized advisors that claimed that farmers were dependent on external advice.

Farmers' Discourse F1: "Awareness"

Eight of 26 farmer respondents (30.8%) contributed to the "Awareness" discourse, which included four chicken farmers, three duck farmers, and one free-grazing duck farmer. They reported that they never used antimicrobials as the first choice of treatment if they did not know the reason why their birds were sick (3, −3), and reported that they never used antimicrobials for disease prevention (14, +2). These respondents knew that overuse of antimicrobials leads to their loss of effectiveness (44, +3). They believed that improper AMU might cause sudden death in some cases (40, +3). Furthermore, these farmers appreciated the importance of biosecurity, and completely disagreed with the notion that when flocks are given antimicrobials, there was no further need for other disease

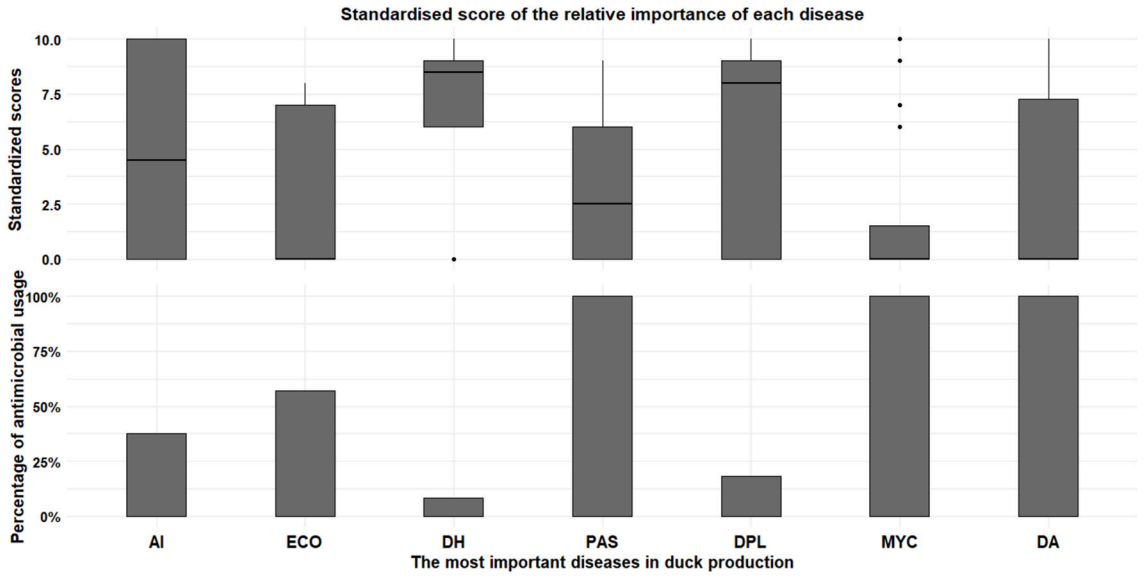

FIGURE 3 | Classification of the most important diseases (top) and frequency of AMU conditional to disease present in their farm (bottom) for duck farms. AI, Highly Pathogenic Avian Influenza; ECO, *Escherichia coli;* DH, Duck Hepatitis; PAS, Pasteurellosis; DPL, Duck Plague; MYC, Mycoplasmosis; DA, Duck Aspergillosis.

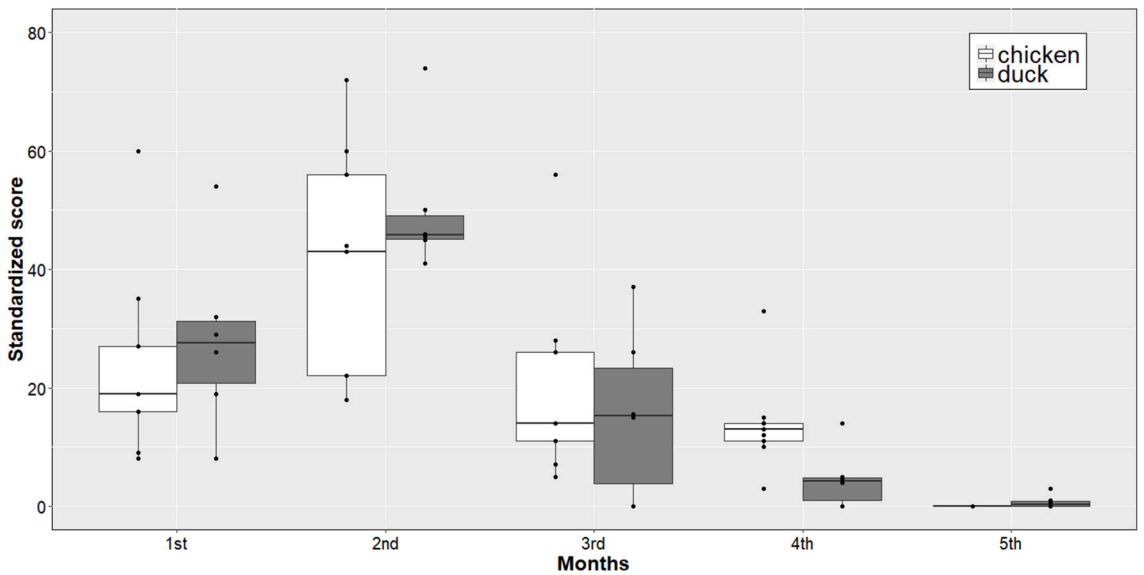

FIGURE 4 | Stakeholders' opinion about source of advice to farmers on AMU. White: Farmers' source of advice (farmers' opinion); Gray: Farmers' source of advice (Farmers' advisors opinion); VDSO, Veterinary drug shops owners; G.V/CAHW, Government Veterinary/Community Animal Health Workers; SPPC, Salespersons of pharmaceutical companies; SPFC, Salespersons of feed companies; FC, Farmer colleagues; FKE, Own farmer' knowledge/experience.

control methods (23, −3; 24, +2). They trusted the advice of government veterinarians/CAHWs about AMU (21, +2), and they did not seek advice on AMU from neighboring farmers (19, −3). Some also believed that using antimicrobials to treat disease by themselves was more costly than seeking veterinary advice (30, −2).

Farmers' Discourse F2: "Dependency"

Seven farmers (26.9%) (three chicken farmers, one meat duck farmer, and three free-grazing duck farmers) contributed to the "Dependency" discourse. Antimicrobials were always used by these farmers both for prevention and treatment of disease (13, −3; 14, −3; 15, −3). Even if flocks were kept in conditions of high biosecurity, they would still use antimicrobials for prevention (24, −3), since AMU gave them a sense of security (1, +2). They also reported that they medicated their bird as soon as they heard of a disease outbreak spreading in their area (17, +3). They also perceived that the costs of antimicrobials were too high relative to overall total production costs (36, −2). However, when asked about how a potential three- to four-fold increase in price would

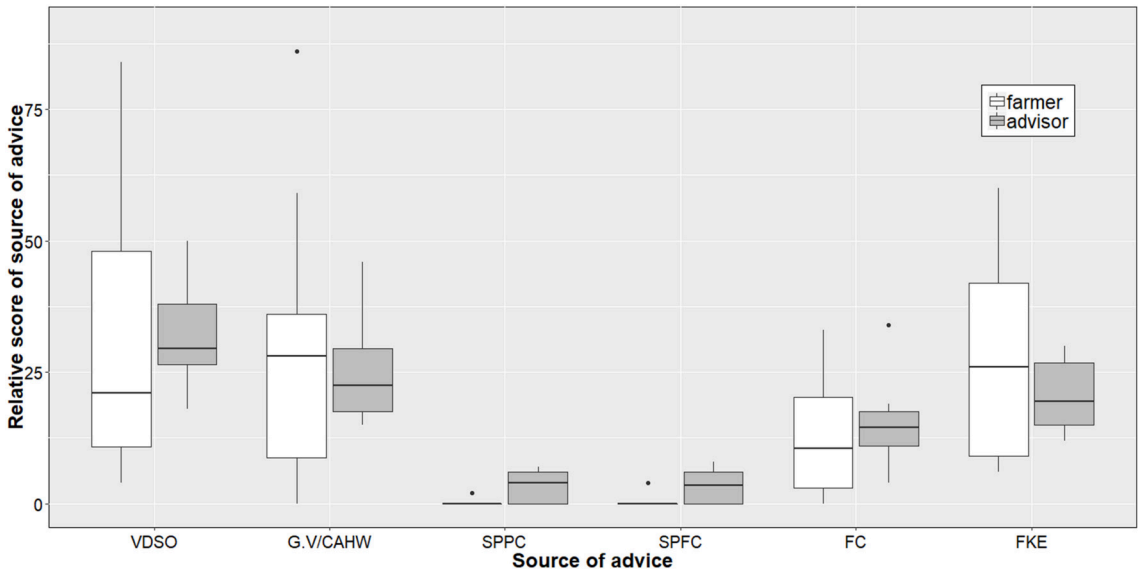

FIGURE 5 | Timing of AMU during production cycle.

affect their AMU practices, these farmers stated that they would not change their current AMU practice (41, −2). They trusted the advice on AMU from government veterinarians/CAHWs (21, +1). They believed that antimicrobials offered by veterinarians were of good quality (6, +2), and were more willing to allow veterinarians treat their sick flocks rather than undertaking this task by themselves (31, −2).

Farmer's Discourse F3: "Initiative"

The "Initiative" discourse corresponded to six respondents (23.1%) owning larger flocks (two chicken farmers, one meat duck, and three free-grazing duck farmers with more than 500 heads each). Farmers assigned to this discourse frequently relied on antimicrobials for prevention and treatment of disease in their flocks (13, −3; 14, −3). They felt secure when their flocks were given antimicrobials for prevention (2, +3). They reported that in spite of good farming practices, antimicrobials were still necessary as prophylactic and therapeutic agents (26, +3). They relied on their own knowledge and experience in terms of choosing the most appropriate antimicrobial product to treat their flocks (8, +2; 31, +2). They expressed a lack of trust in private veterinary, veterinary drug shop owners (22, −2).

Farmer's Discourse F4: "Imperfectness"

The "Imperfectness" discourse was assigned to five respondents (19.2%) (two chicken farmers, two meat duck farmers, and one free-grazing duck farmer) who generally trusted the advice of government veterinarians/CAHWs (21, +3). They believed that antimicrobials were not needed if birds were raised in conditions of good biosecurity (24, +3). They followed the recommended full treatment course indicated in the product label (25, −3). They believed that preventing disease by vaccination would be more cost-effective than medicated sick birds (42, −3). However, if their flocks became sick they applied antimicrobials to the whole flock (including healthy-looking birds) as their first choice (3, +2; 16, −2). They expressed relieve after their sick flock became treated with antimicrobials (1, +2).

Advisors' Discourse A1: "Negativism"

Eight respondents in this group (36%) (two veterinary drug shop owners, six government veterinarians/CAHWs) believed that farmers continued to have considerable misunderstandings about AMU. They assumed that farmers resorted to use antimicrobials as their first choice when dealing with disease problems (3, +3), farmers made their decisions about AMU without any input from external advisors (18, +3; 31, +3). AMU gave farmers a sense of security, reducing their stress and increasing their confidence in their production system (1, +2; 2, +2). Most farmers used antimicrobials for disease treatment and prevention, as well as for growth promotion (13, −3; 14, −3; 15, −3). They considered that farmers never followed the recommended dosing instructions in the label when treating animals (11, +2). When a part of flock was sick, instead of placing segregating it (i.e., in a sick pen), farmers would rather give antimicrobials to the whole flock in an attempt to prevent disease spreading (16, −3).

Advisors' Discourse A2: "Hopefulness"

The advisors' "Hopefulness" discourse described the actions and beliefs of eight respondents (36%) (five veterinary drug shop owners, three government veterinarians/CAHWs). Respondents in this discourse believed that farmers would not use antimicrobials for prevention if good biosecurity were applied in their poultry farming (24, +2; 17, −2). These advisors believed that farmers knew that overuse and misuse of antimicrobials resulting in their loss of effectiveness (i.e., AMR) (44, +3), contributing to decreasing animal productivity (39,

TABLE 2 | Opinions about positive and negative aspects with regards to AMU reported in farmers' CI (16) and in farmers' advisors' CI (10).

	Farmers' (CI = 16) opinions				Farmers' advisors' (CI = 10) opinions			
	Positive aspects	**%**	**Negative aspects**	**%**	**Positive aspects**	**%**	**Negative aspects**	**%**
Using antimicrobial	Disease treatment	100	Increases production costs	68.8	Disease prevention	80.0	Treatment failure	70.0
	Disease prevention	87.5	Reduces productivity	62.5	Avoids mortality	80.0	Reduces productivity	50.0
	Reduces mortality	50.0	Increases feed costs	50.0	Disease treatment	70.0	Antimicrobials residues in meat and egg	40.0
	Keeps flocks healthy	37.5	Treatment failure	37.5	Increases income	30.0	Increases production costs	40.0
	Increases income	6.3	Increases labor costs	25.0			Increases feed costs	40.0
			Risk of using counterfeit drugs	18.8				
			Antimicrobials residues in meat and egg	18.8				
Not using antimicrobial	Saves money through decreases costs of production	50.0	Increases mortality due to disease	62.5	Saves money through decreases costs of production	50.0	Increases mortality due to disease	60.0
	Increases productivity	31.3	Weakens the immune system	6.3	Increases meat and egg quality	40.0	Reduces productivity	10.0
	Provides safe products	31.2			Flock grows faster	20.0	Reduces income of vet drug-shop owners	10.0
	More time for other activities	18.8			No antimicrobials residues in meat and egg	10.0	Unable to cure diseases	10.0

+3). They believed that farmers mostly trust in the advice from government veterinarian/CAHWs (21, +2).

Advisors' Discourse A3: "Advice dependency"

The "Advice dependency" discourse was attributed to six advisors (23.1%) who claimed that farmers generally had poor knowledge of good AMU practices, and were dependant on advice provided by private veterinarians and veterinary drug shop owners (22, +3). This group believed that farmers used antimicrobials to both prevent and treat disease in their flocks (13, −3; 14, −3). They believed that farmers would use antimicrobials over the entire production cycle even if only a proportion of birds in the flocks were sick (16, −2), and would also use them when hearing of a disease outbreak in the surrounding area (17, +2). They also thought that farmers would decrease AMU when the price of antimicrobials increased (41, +2).

Consensus Points From Q-Sorting Analysis

Among farmers, only one consensus point was found: they all believed that the cost of antimicrobials was more expensive than the cost of other biosecurity methods as currently applied in their farms (Statement 38). All advisors agreed on the following statements: (a) The cost of treating flocks using antimicrobials was more expensive than the cost of using vaccines for prevention (Statement 35); (b) Farmers would use antimicrobials as first choice when dealing with their sick flock over other practices such as segregating sick birds and early mortality culling (Statement 12); (c) They all had no opinion about the price of antimicrobials in relation to the total production costs (Statement 36); and (d) They had no knowledge about the timing when antimicrobials are most commonly used by farmers (Statement 27).

DISCUSSION

Through the Q-sorting we identified four distinct attitudes (discourses) on AMU among farmers. They were all relatively evenly distributed, with each of these accounting for 19.2–30.8% of farmers investigated. The "Awareness" discourse was the most prevalent (30.8%). Farmers assigned to this discourse were aware of the limitations and issues regarding AMU/AMR, and reported never using antimicrobials to prevent disease. On the other extreme, a group of farmers (26.9%) were assigned to a discourse ("Dependency") that reflected total dependency on antimicrobial for raising their flocks.

In contrast with traditional questionnaire-based interviewing methods, PE allows farmers to freely explore the topics by themselves, and therefore it was considered to be most appropriate in this setting. This study fulfilled the criteria outlined for PE studies described by Catley et al. (12): (1) active involvement of respondents allowing them to express their opinions and perceptions; (2) local knowledge about concrete problems collected from CI was used to generate statements in Q-sorting interviews (bottom-up approach); (3) the collected data was triangulated during interviews and between interviews with the help of open-ended questions. However, the choice of specific themes proposed in the CI phase was naturally influenced by

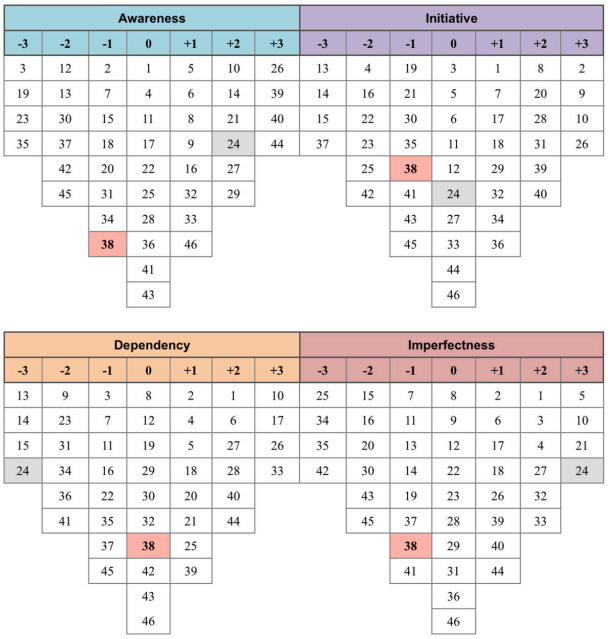

FIGURE 6 | Four discourses summarizing farmers' opinions. Number from +3 to −3: Score in one discourse; Number in red cells: Consensus statements; Number in gray cells: Distinguished statements (see **Supplementary Table 1** for detailed information).

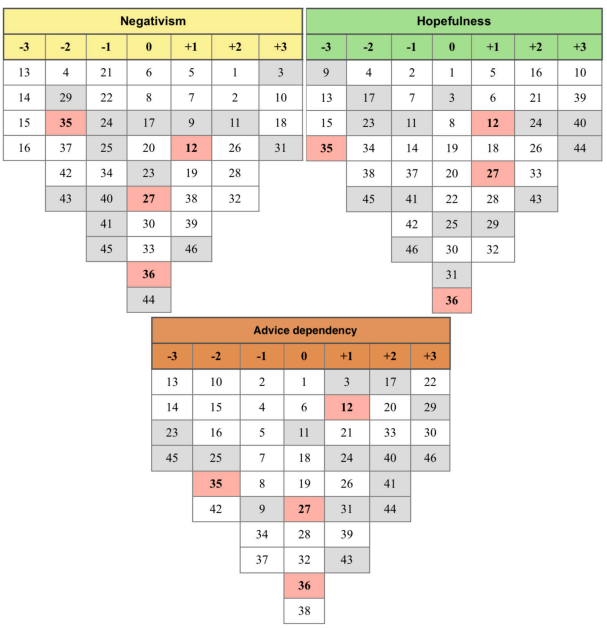

FIGURE 7 | Three discourses summarizing farmers' advisors' opinions. Number from +3 to −3: Score in one discourse; Number in red cells: Consensus statements; Number in gray cells: Distinguished statements (see **Supplementary Table 1** for detailed information).

the experience and knowledge of the researchers on AMU/AMR. Farmers were selected from a farm census maintained and updated annually by SDAH-DT. The census is not balanced with regards to gender, since the overwhelming majority of registered farm owners were male. The reason is that in rural Vietnamese households, the named farm owner is typically the husband, even if the responsibility for tending poultry flocks often lies within the wife. During the initial telephone call to the owner as part of the recruitment process, we aimed to achieve a more balanced sample by inviting the person (male or female) directly responsible for tending the poultry flocks. In spite of that, 90% of the participating farmers were male. We believe this is due to the fact that in Vietnamese culture it is normally the male who liaises with external agents. Because of this, we might not have captured all of the women's opinions related to the study research questions. Another potential bias might be an under-representation in our sample of part-time farmers or farmers having other occupations in addition to tendering poultry. We used the same 46 statements to investigate attitudes on AMU in both farmers and their advisors. Since some farmers' advisors had limited knowledge/experience on AMU, this might have been a source of bias in the Q-sorting interviews. Therefore, some of their opinions about farmers' attitudes may be more a reflection of circulating views than actual hands-on advisory experience.

Farmers reported that the highest amounts of antimicrobials were used during the first half of the production cycle. This corresponds to the brooding and early grow-out periods. This period often involves changes in housing and feeding conditions and is perceived to be the period when flocks are at their highest risk of disease. Increased risk of disease was also reported during at two critical time points: during vaccination against viral diseases to control secondary bacterial infections, and during transition from the dry to the rainy season. Farmers believed that using antimicrobials during this period helped them reduce their anxiety about the risk of diseases. Keeping the flock healthy and maximizing number of live birds sold to the market (outputs) whilst lowering the input costs as much as possible are the two main targets of poultry farmers (30). Therefore, in the eyes of many farmers, antimicrobials are seen as part of "good farming practice." Because of this, they might also neglect other disease control measures (31).

All farmer participants agreed that the costs incurred in AMU were higher than the cost of biosecurity and disease control methods as were currently implemented in their flocks (Statement 38). They however recognized that should they upgrade biosecurity and other disease control methods, this would eventually lead to greater reductions in the incidence of disease and therefore this would result in reduced need of antimicrobials. From our observations, biosecurity methods implemented by most farmers in the area typically consist of keeping pens visibly clean and regularly applying disinfectants. Most farmers are also regularly supplied with disinfectants and HPAI vaccines free of charge by the veterinary authorities. Farmers often think that disease control programmes supported by the veterinary authorities are crucial in reducing the risks to their flock. A recent study showed that Vietnamese cattle farmers felt more secure after taking part in an official vaccination campaign (17). However, it has been shown that even well-established vaccination campaigns such as HPAI in poultry may in fact provide limited protection against circulating viruses (32).

Therefore, the provision of vaccines by the veterinary authorities may have a negative impact by creating a false sense of security.

The fact that some farmers in the area were prepared to accept a three- to four-fold increase in the price of antimicrobials suggests that there is a potential for revising pricing policies, increasing them to deter AMU in situations when antimicrobials are not strictly necessary. However, rapid increases in prices could potentially result in the creation of a black market for antimicrobials.

Compared with private veterinarians and veterinary drug shop owners, government veterinarians/CAHWs were ranked by famers as a more trustworthy source of information on AMU (**Figure 3**). Farmers also reported that they had more regular contact with private veterinary drug shop owners than with other stakeholders. In each commune in the area, there is typically only one or two government veterinarian/CAHW, compared with three-six veterinary drug shop owners. Veterinary drug shop owners have a vested interest in antimicrobial sales. Many smallholder farmers tend to rely on their own experience with regards to AMU ("Initiative" discourse). Participants in this discourse would just ask for advice when they encounter more serious disease. This attitude was closely linked to large (>500 heads) free-grazing (mobile) duck flocks that typically travel long distances to graze on paddy fields. Farmers of these flocks feel they need to be prepared to administer antimicrobials should their flocks become diseased in locations far from their "local" veterinary drug shops.

The lack of understanding of animal health advisors on poultry farm-level economics is of concern. The advisors' belief that poultry production costs could be easily reduced by adopting alternative disease control practices contrasts with the farmers' understanding of the costs of AMU vs. biosecurity and vaccination. This lack of agreement is an area for education and training policy, whilst requiring further research on the economics of AMU and alternative animal health interventions.

CONCLUSIONS AND RECOMMENDATIONS

A combination of PE and Q-sorting approach provided meaningful insights into the attitudes of the different stakeholders involved in the procurement and usage of antimicrobials in poultry farming. Through the study of 203 participants in Dong Thap province, we characterized the purpose of AMU (treatment and prevention), the timing associated with higher AMU levels (first half of the production cycle), and the cost of AMU (cheaper than other biosecurity methods). Farmers were aware of good husbandry practices (including good biosecurity) as effective disease control tools. However, these practices were regarded as expensive alternatives to AMU, and their implementation would require sustained training efforts. Given that farmers have relatively greater trust of official government veterinarians, we recommend reinforcing their advisory role in order to counteract the influence of veterinary drug shop owners (currently the first point of contact to farmers). From an educational perspective, veterinarians, and animal health advisors need guidance on farm-level economics of poultry farming and a better understanding of costs. This reinforced advisory capacity should focus on improving overall farming practices whilst discouraging prophylactic AMU. Given the complexity and diversity of poultry production systems in the Mekong Delta region of Vietnam, we recommend scaling up research on socio-economic factors driving AMU in small-scale farms in the region. Furthermore, the clear gender imbalance evidenced in our study population suggests that more research is needed to understand the perceptions and views of female Vietnamese farmers with regards to AMU.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

The ViParc project has been granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Ref. 5121/16).

AUTHOR CONTRIBUTIONS

HD, DT, FG, JC-M, and JR designed the study, contributed to the analyses, and drafted the manuscript. VD, VN, and TB designed the data collection instrument and drafted the manuscript. CR aided in reviewing results and provided discussion comments. AB and GT reviewed the results and drafted the manuscript. The manuscript has been read and approved by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00084/full#supplementary-material

Supplementary 1 | Checklist used for CI-participatory epidemiology approach.

Supplementary 2 | Script for Q-sorting interviews analysis run in R program.

Supplementary Table 1 | List of statements used in this study and their score according to each factor/discourse after Q-sorting analysis.

Supplementary Table 2 | Raw data of Q-sorting interviews.

Supplementary Figure 1 | Flow chart of study steps.

Supplementary Figure 2 | Flow chart of statistical analyses steps.

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Mortality, disease and associated antimicrobial use in commercial small-scale chicken flocks in the Mekong Delta of Vietnam

Juan Carrique-Mas[a,b,*], Nguyen Thi Bich Van[a], Nguyen Van Cuong[a], Bao Dinh Truong[c], Bach Tuan Kiet[d], Pham Thi Huyen Thanh[d], Nguyen Ngoc Lon[d], Vu Thi Quynh Giao[a], Vo Be Hien[d], Pawin Padungtod[e], Marc Choisy[a,f], Erry Setyawan[g], Jonathan Rushton[h], Guy Thwaites[a,b]

[a] Oxford University Clinical Research Unit, Vietnam
[b] Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, Oxford University, Oxford, United Kingdom
[c] Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh, Vietnam
[d] Sub-Department of Animal Health, Cao Lanh, Dong Thap, Vietnam
[e] Emergency Center for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations, Green One UN House Building, 304 Kim Ma, Hanoi, Vietnam
[f] MIVEGEC, IRD, CNRS, University of Montpellier, France
[g] Food and Agriculture Organization of the United Nations, Jakarta, Indonesia
[h] Institute of Infection and Global Health, University of Liverpool, United Kingdom

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ABSTRACT

Raising chickens in small-scale flocks following all-in-all-out management is common in the Mekong Delta of Vietnam. These flocks represent an intermediate category between backyard and intensive (industrial) farming systems. However, little is known about the occurrence and burden of disease and/or mortality in such flocks, and their potential association with antimicrobial usage (AMU). We investigated mortality, disease and weekly antimicrobial use (AMU) in 124 cycles of meat chicken flocks raised in 88 farms in the Mekong Delta of Vietnam (with a median cycle duration of 18 weeks [inter-quartile range IQR 17–20]). We visited each farm 4 times per cycle to review data collected weekly by the farmers on clinical signs, mortality, and AMU. The overall probability of disease and AMU were 0.31 (95% CI 0.29–0.32) and 0.26 (95% CI 0.24–0.28), respectively. The average weekly incidence of mortality was 2.6 (95% CI 2.2–3.0) per 100 birds. Both the probabilities of a flock experiencing disease and mortality, as well as of using antimicrobials decreased with the flock's age. However, mortality peaked at the 5–10 week period. The only significant explanatory factors associated with presence of disease was the stage of production ≥5 weeks (protective) (OR ≤ 0.51). Factors independently associated with AMU ($p < 0.05$) were: (1) Number of chickens (log) (OR = 1.46), (2) Stage of production ≥5 weeks (OR ≤ 0.67) (protective), (3) Cao Lanh district (OR = 2.23), (4) Density of veterinary drug shops at commune level (log) (OR = 1.58), and (5) Disease in flocks (OR = 1.80). Factors independently associated with overall increased weekly incidence of mortality ($p < 0.05$) were: (1) High level of education attainment (secondary education or higher) (Hazard rate Ratio [HR] = 1.70), (2) number of chickens (log) (HR = 1.39), and (3) Stage of production > 5 weeks (HR ≤ 2.14). In flocks reporting disease, AMU significantly reduced the incidence of mortality (HR = 0.90). These results confirm an exceptionally high mortality in chicken flocks in the area, jeopardizing the profitability and sustainability of these small-scale farming systems. The data also suggest an association between nearby access to antimicrobials and AMU, and a high correlation of AMU over consecutive cycles. The atomized farming landscape of the Mekong Delta, the high incidence of disease and mortality, and the unrestricted and easy access to antimicrobials present major challenges to the implementation of policies aimed at AMU reductions.

* Corresponding author at: Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, 764, Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam.
E-mail address: jcarrique-mas@oucru.org (J. Carrique-Mas).

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1. Introduction

With over 100 million tons produced per year (2014) chicken meat is the second most common animal food commodity worldwide (FAO, 2017). In low- and middle-income countries, chickens are often raised in backyard and small-scale and flocks, supporting rural livelihoods by providing animal protein and nutrients (meat and eggs), as well as manure and feather bio-products. In many countries chicken meat is also central to festivities and traditional ceremonies (Alders and Pym, 2009; FAO, 2010). Therefore, high levels of disease and mortality in small-scale farms pose major constraints to the livelihoods of large numbers of poor people worldwide, and infectious diseases are thought to be responsible to a large extent (Bell, 2009). Over recent years, more and more farms in the Mekong Delta have been upgrading their production capacity, transitioning from 'backyard' to confined housing and flock management using all-in-all-out principles. Much of the published research on poultry diseases in southeast Asia has consisted on the detection and characterization of single bacterial and viral pathogens (Jonas et al., 2001; Eagles et al., 2009; Chukiatsiri et al., 2012). In Vietnam, research has overwhelmingly focused on Highly Pathogenic Avian Influenza (HPAI) (Lee et al., 2015; Nguyen et al., 2017), due to its high pathogenicity in poultry, and its pandemic potential. Although HPAI is still endemic in the Mekong Delta of Vietnam, large outbreaks of the disease are now less common compared with the 2003–2006 period, when the HPAI H5N1 epidemic was first reported (Anon, 2018; FAO, 2018; Meyer et al., 2018). In addition to HPAI, several viral poultry diseases, such as Newcastle Disease (Choi et al., 2014), Infectious Bursal Disease (IBD), and Infectious Bronchitis (IB) (de Witt et al., 2010) are all suspected to be widely circulating in Vietnam, and therefore vaccination programmes largely focus on these diseases (Bui et al., 2001). However, no data on circulation/incidence of these viral diseases, as well as major bacterial diseases and coccidiosis in the area are available.

Antimicrobial use (AMU) in animal production is a key driver of antimicrobial resistance (AMR) worldwide (O'Neill, 2015). It has been estimated that, worldwide, on average, 148 mg of antimicrobial active principle are used to raise 1 kg of live chicken, closely following antimicrobial use in pig production (172 mg) (Van Boeckel et al., 2015). In the Mekong Delta region of Vietnam high levels of AMU in chicken production have been reported (˜260 mg kg of chicken, excluding medicated feed) (Carrique-Mas et al., 2014; Trung et al., 2015). These quantities are, in part, due to the widespread circulation of infectious diseases, which in turn is associated with deficient levels of sanitation and health management – often termed 'poor farm biosecurity' (Hong Hanh et al., 2007b). In 2015 there were 277 million chicken heads in Vietnam, ˜20% of which were in the Mekong Delta (FAO, 2017). The number of households engaged in small-scale poultry production in the country is estimated in about 8 million, with an average flock size of ˜32 birds (Burgos et al., 2007). Small-scale poultry production plays an important role in rural areas, contributing to 19% of household income (Desvaux et al., 2008). In spite of the importance of small-scale chicken farming in Southeast Asia, there is limited information on disease patterns and mortality in these systems. To address this critical gap, we investigated a large sample of chicken farms with the following aims: (1) to quantify mortality; (2) to characterise disease patterns; and (3) to investigate associations between AMU, disease and mortality in flocks. The knowledge on disease and associated mortality in smallholder poultry flocks is an important and necessary step to improve farm management and adopt effective control measures to improve farm productivity and help reduce the farmer's reliance on antimicrobials.

2. Materials and methods

2.1. Study location and farm recruitment

This study was carried out on farms raising chickens for meat with a flock capacity of > 100 birds (case definition) in the districts of Cao Lanh and Thap Muoi within Dong Thap province (Mekong Delta region of Vietnam), as part as the baseline phase of a research project (Carrique-Mas and Rushton, 2017). These small-scale commercial flocks lie between 'backyard' flocks and intensively managed 'industrial' systems. These flocks roughly correspond to FAO Sectors 2 and 3 (between 50 and 2000 birds, with feed and water supplied to the birds) (FAO, 2010). Meat chicken flocks are typically based on slow-growing local breeds (4–5 months to reach a market weight of 1.6-2.0kg), raised as single age and confined in a dedicated house/pen. The chickens are kept at ambient temperature, except for the brooding period (first 4 weeks), where chicks receive additional heating. However, in some cases, chickens may have some access to grazing areas within the farm. In some instances, farmers may purchase day-old chicks from several sources over the first few weeks, and birds are often sold over a period of 1–4 weeks. All feed- and water-dispensation is manual, with flocks being predominantly raised on commercial feed. A total of 207 farmers randomly selected from the census were contacted by letter by the veterinary authorities (sub-Department of Animal Health and Production of Dong Thap, SDAHP). A meeting was held with 199 attending farmers (96%), where the project aims and methods were presented. Farmers where asked to contact project staff as soon as they restocked with day-old chicks. From each study farm, chicken flocks (defined as a group of birds raised together in the same building) that met the case definition and had completed at least one full production cycle over the time frame of the study were included (study flocks).

Of 106 farmers that met the case definition that planned to restock within 4 months of the meeting, 88 agreed to participate in the study (84% participation). These 88 farms were investigated over a total of 124 fully completed production flock cycles from October 2016 to March 2018 (54 farms over 1 cycle, 32 over 2, and 2 over 3 consecutive cycles). Farm visits were carried out by veterinarians affiliated to the SDAHP. Farm location is shown in Fig. 1.

2.2. Data collection

Farmers were provided with a notebook laid out as two A4 sides by week, and were instructed to note down the following information related to their flock: (1) Movements of chickens in and out of their farm (i.e. numbers of birds bought, sold and dead on farm); (2) Any observed clinical signs, including: malaise (ruffled feathers, prostration), signs of respiratory infection (sneezing, coughing, wheezing, nasal secretion), enteric infection (diarrhoea), signs of central nervous system (CNS) disorder (ataxia, torticollis, circling) or other signs (i.e. lameness); (3) Use of health-supporting products (including vaccines). Farmers were instructed to keep bottles and containers of all health-supporting products used on their flocks. Farms were visited four times during each production cycle to review the data. On the first visit, generic data on the farmer, the farm and the chicken house were collected. On this first visit farmers were also trained by project veterinarians to recognize the main clinical signs, supported by a Vietnamese text book on poultry diseases that contains a description and visual images of the most common signs of chicken flocks where appropriate. This training was repeated several times on subsequent visits to the farms. Data on flock-related variables were collected on subsequent visits. Visiting veterinarians reviewed the labels of all commercial products given to the chickens and determined which products contained antibacterial antimicrobial active ingredients.

2.3. Statistical analyses

'Disease' was defined for a flock on a given week when signs of disease were observed in at least 5% of the birds in the flock. The probability of 'Disease', and the probability of using antimicrobials was computed for each week of the production cycle, with the total number of flocks observed on any given week taken as the denominator. The

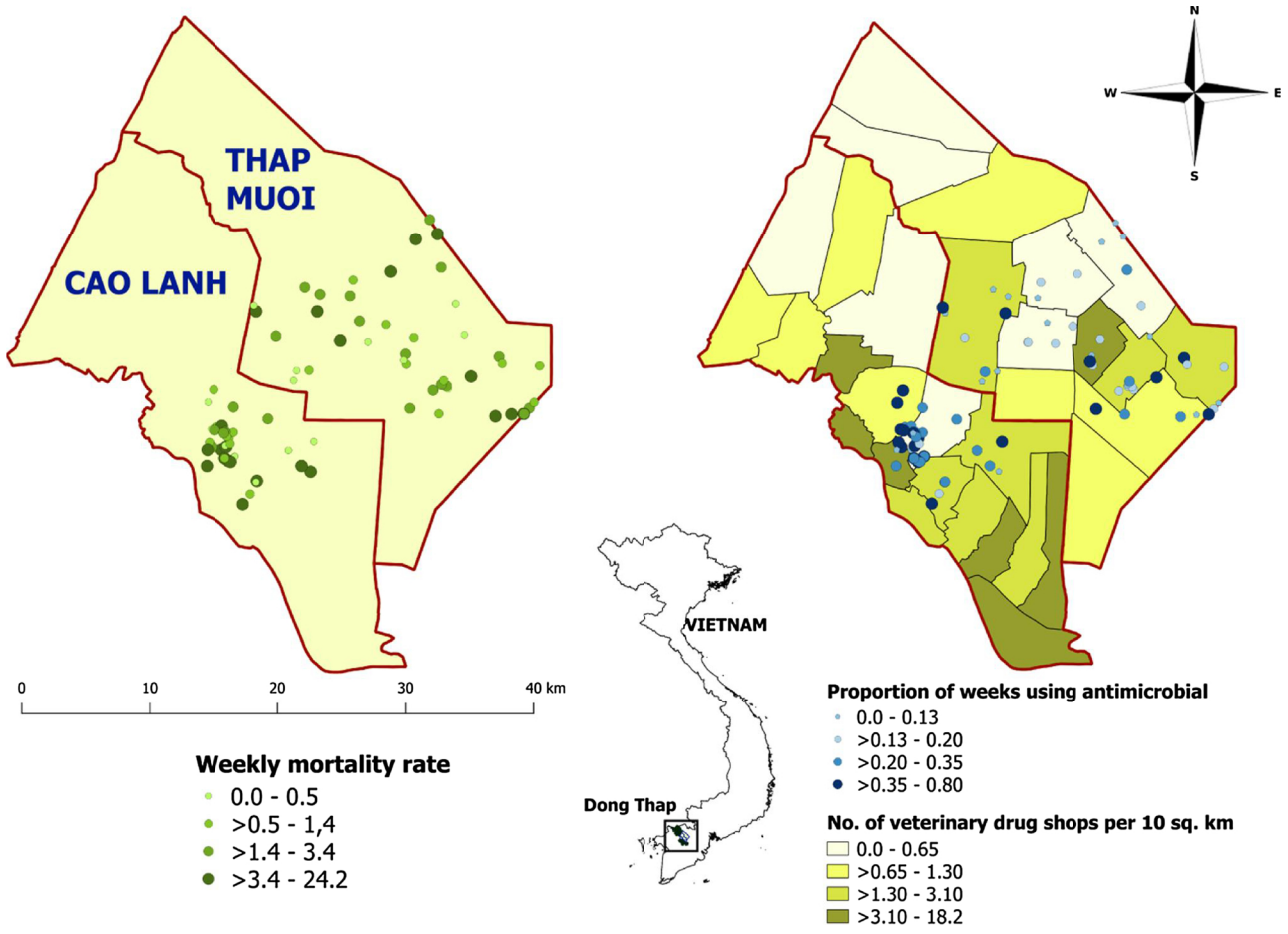

Fig. 1. Location of study farms (n = 88) in the two study districts (Cao Lanh and Thap Muoi) within Dong Thap province. The average weekly incidence of mortality (per 100 birds), as well as the proportion of weeks that farmers used antimicrobials, and the density of veterinary drug shops are displayed.

'weekly (cumulative) incidence of mortality' was calculated for all study flocks by dividing the total number of chickens dying each week by the total number of chickens present on farms at the beginning of each week. Any chickens purchased halfway through the week were included in the denominator for the calculation of the following week period. The flock cycle (cumulative) incidence of mortality was calculated for each flock cycle by dividing the total number of birds dying from restocking to sale, divided by the total size of the flock at restocking. The weekly incidence of mortality was modelled using a Poisson model, with 'Farm' included as a random effect, and the size of the flock at the beginning of the week (log) as the offset. The association between farmer, farm, and flock characteristics (outcomes) and the variables 'Disease' (Yes/No)' and 'Antimicrobial Use (AMU)' (Yes/No) (responses) were investigated by building multivariable logistic regression models, with 'Farm' modelled as a random effect. The following independent variables were investigated: Farmer-related: (1) Farmer/farm owner's gender, (2) Age of farm owner (Years) (log), (3) Highest level of education attainment of farmer/farm owner, (4) Experience in chicken farming (Years) (log); Farm-related: (5) Type of chicken house, (6) Presence of chickens other than the target flock in the farm, (7) Presence of other poultry species other than chickens; Flock-related: (8) Number of chickens, (9) Week of production; Geographical variables: (10) District (Cao Lanh/Thap Muoi), (11) Number of chickens per km^2 by commune, and (12) Number of veterinary drug shops per km^2 by commune. Variables were ranked by their degree of significance, and were included in the models using a stepwise forward approach, starting with the ones with the lowest p-value obtained from the likelihood ratio test (LRT) comparing a model with and without the variable. The variables The variables 'Disease' and 'Mortality' (Yes/No) were investigated in the model with AMU as response variable to investigate to what extent AMU was a function of health events on farm. Variables with $p \leq 0.05$ from the LRT were retained in final multivariable models. The Intra-cluster Correlation Coefficient (ICC) was calculated for the two final multivariable logistics models to investigate the percent of the total variation associated with the clusters (farms). The potential impact of AMU on the weekly incidence of mortality given disease was investigated by fitting a model on a subset of data corresponding to weeks where disease was reported, with weekly mortality as response, and the variables AMU (Yes/No) and clinical signs reported (respiratory, diarrhoea, CNS and malaise) as explanatory variables. The correlation between AMU in weeks with and without disease, as well as the correlation between AMU over subsequent cycles of production was estimated using the Pearson correlation coefficient. All statistical analyses were performed using the lme4 and MASS packages within R statistical software (http://www.r-project.org).

2.4. Ethics

This study was part of the ViParc project, which was granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Ref. 5121/16) and by the local authorities (People's Committed of Dong Thap province) (May 2016).

Table 1
Unadjusted weekly probability of disease and/or mortality and antimicrobial use, and weekly incidence of mortality (per 100 birds) by study variables in chicken flocks for 124 cycles of production (Dong Thap, Mekong Delta, Vietnam).

	No. farms (*flocks) (No. weeks)	Disease (Y/N)		Weekly incidence of mortality (per 100 birds)		Antimicrobial use (Y/N)	
		Prop.	95% CI	Mean	95% CI	Prop.	95% CI
Farmer's gender							
Male	11 (337)	0.32	0.30-0.34	2.60	2.14-3.05	0.27	0.25-0.29
Female	77 (1890)	0.23	0.19-0.27	2.48	2.03-2.94	0.21	0.16-0.25
Farmer's age							
Up to 45	44 (979)	0.31	0.28-0.38	2.90	2.22-3.58	0.27	0.24-0.30
Over 45	44 (1248)	0.30	0.28-0.33	2.33	1.83-2.83	0.26	0.23-0.28
Farmer's highest education attainment							
Primary school	22 (638)	0.25	0.21-0.28	1.56	1.02-2.11	0.27	0.24-0.31
Secondary school	36 (904)	0.32	0.29-0.35	2.35	1.76-2.93	0.26	0.23-0.29
High school	25 (544)	0.34	0.30-0.38	3.62	2.58-4.67	0.28	0.24-0.32
Post high school	5 (141)	0.35	0.28-0.43	4.67	2.37-6.98	0.20	0.13-0.26
Farmer's experience in chicken farming (years)							
0-1.5	20 (506)	0.28	0.24-0.32	2.96	2.02-3.91	0.25	0.22-0.29
1.6-2.3	32 (745)	0.31	0.28-0.34	2.71	1.94-3.48	0.27	0.24-0.30
2.4-3.5	21 (595)	0.34	0.30-0.38	2.22	1.57-2.87	0.25	0.21-0.28
3.6-11.0	15 (381)	0.28	0.24-0.33	2.39	1.46-3.33	0.28	0.24-0.33
Type of chicken house*							
Solid ground	72 (1873)	0.32	0.30-0.34	2.61	2.15-3.62	0.26	0.24-0.28
Stilts on ground	5 (79)	0.14	0.06-0.22	2.16	0.18-4.13	0.30	0.20-0.40
Stilts on water	10 (265)	0.27	0.22-0.33	2.21	1.36-3.06	0.26	0.21-0.31
Solid and stilts	1 (10)	0.30	0.02-0.58	10.52	0.0-30.0	0.50	0.19-0.81
Presence of chickens other than the target flock*							
No	57 (1015)	0.28	0.25-0.30	2.76	2.09-3.44	0.26	0.24-0.29
Yes	67 (1212)	0.33	0.30-0.36	2.43	1.93-2.93	0.26	0.24-0.29
Presence of non-chicken poultry species*							
No	55 (839)	0.30	0.27-0.33	2.78	2.18-3.38	0.27	0.24-0.30
Yes	69 (1389)	0.31	0.28-0.34	2.35	1.80-2.89	0.26	0.23-0.28
No. chickens restocked*							
100-199	22 (372)	0.20	0.16-0.24	1.82	1.25-2.39	0.23	0.19-0.27
200-299	30 (527)	0.28	0.24-0.32	2.02	1.22-2.83	0.24	0.21-0.28
300-499	38 (692)	0.31	0.28-0.35	3.40	2.52-4.28	0.26	0.23-0.29
500+	34 (636)	0.38	0.34-0.42	2.60	1.83-3.37	0.30	0.27-0.34
Week of production (age of flock)*							
1-5	124 (494)	0.48	0.44-0.53	2.42	1.95-2.88	0.39	0.33-0.42
> 5-10	124 (607)	0.36	0.32-0.39	3.66	2.62-4.70	0.29	0.25-0.33
> 10-14	116 (457)	0.22	0.18-0.25	1.96	1.18-2.74	0.21	0.17-0.24
> 14-26	111 (545)	0.09	0.06-0.11	2.09	1.22-2.95	0.05	0.03-0.06
District							
Thap Muoi	46 (1282)	0.29	0.26-0.31	2.46	1.98-2.93	0.20	0.18-0.22
Cao Lanh	42 (945)	0.33	0.30-0.36	2.75	2.03-3.46	0.35	0.32-0.38
Commune density of chickens (per km^2)*							
1-320	46 (1119)	0.28	0.25-0.31	2.68	2.12-3.25	0.21	0.18-0.23
> 320	42 (1028)	0.33	0.30-0.36	2.46	1.87-3.05	0.33	0.30-0.358
0-1	50 (1226)	0.29	0.26-0.32	2.78	2.21-2.35	0.28	2.21-3.35
Commune density of veterinary drug shops (per $10km^2$)							
> 1	38 (1001)	0.31	0.29-0.34	2.34	1.76-2.92	0.24	1.76-2.92

3. Results

3.1. Study farms

The median flock size at restocking was 303 birds [inter-quartile range (IQR) 202–500]. The unadjusted prevalence of disease and/or mortality, AMU and the average mortality (per 100 birds) (per week) by levels of the variables investigated are shown in Table 1. The median duration of one production cycle was 18 [IQR 17–20] weeks. Most (81.8%) flocks were raised on houses/pens on solid ground, whereas others were housed on stilts, either over a canal (8.8%) or on the ground (5.7%). One flock was raised on two types of housing: solid house during the brooding period, and then transferred to a stilted house over a water canal during the grow-out period. A total of 44.3% farms were raising domestic ducks, 12.5% Muscovy ducks, 19.3% pigs and 2.3% cattle at the beginning of the study (Table 2)

3.2. Disease and mortality of chicken flocks

The presence of disease and mortality in a given week in a given flock were highly related ($\chi^2 = 1780$; $p < 0.001$). The mean weekly incidence of mortality in a given week in a given flock was 0.31 (95% CI 0.29-0.32). The highest probability of disease corresponded to the first week of the cycle (0.64; 95% CI 0.55-0.72), and was inversely correlated with the flocks' age in weeks ($r = -0.95$; $p < 0.001$). After 16 weeks, the probability of disease decreased to < 0.1 (Fig. 2a). The (unadjusted) mean weekly incidence of mortality was 0.026 (95% CI 0.022-0.030) (i.e. 2.58 per 100 birds). Mortality was highest during the 5–10 week period, ranging from 0.027 to 0.055 (Fig. 2b). In flocks reporting disease the probability of a bird dying generally increased with the age of the flock (Fig. 2c). The average cumulative mortality over one production cycle was 32.9 per 100 birds (SD ± 30.4), although it was considerably skewed (median 20.9 [IQR 8.9–52.9%]),

Table 2
Risk factors for mortality/disease and antimicrobial use (random effects logistic regression models) and mortality (Poisson models).

	Disease (Y/N)	AMU (Yes/No)		Weekly incidence of mortality (overall)		Weekly incidence of mortality (in weeks reporting disease)
	Univariable OR (p-value)	Univariable OR (p-value)	Multivariable† OR [95% CI]	Univariable HR (p-value)	Multivariable†† HR [95% CI]	Multivariable††† HR [95% CI]
Gender (female)	0.72 (0.210)	0.67 (0.120)		1.14 (0.688)		
Farmer's age (years) (log)	1.03 (0.943)	0.96 (0.900)		0.90 (0.797)		
High school or higher education	1.37 (0.151)	1.07 (0.730)		1.69 (0.030)	1.70* [1.04-2.80]	1.58* [1.03-2.44]
Experience in chicken farming (years) (log)	1.04 (0.841)	1.10 (0.540)		1.24 (0.279)		
Solid ground chicken house (Ref. Stilts)	1.52 (0.136)	0.95 (0.840)		0.76 (0.394)		
Other chicken flock/s	1.30 (0.192)	1.01 (0.970)		0.90 (0.660)		
Other (non-chicken) poultry	1.04 (0.842)					
No. chickens (log)	1.17 (0.236)	1.48 **	1.46‡‡ [0.98-2.17]	1.04*	1.39*** [1.31-1.47]	0.89*** [0.84-0.94]
Week of production (Ref. 1-4)						
5-10	0.51***	0.39 ***	0.67** [0.51-0.90]	1.98***	2.14*** [2.06-2.22]	2.87*** [2.74-3.0]
> 10-14	0.21***	0.22***	0.42*** [0.30-0.59]	1.31***	1.55*** [1.46-1.64]	3.15*** [2.95-3.38]
> 14-26	0.05***	0.03***	0.06*** [0.04-0.10]	1.33***	1.72*** [1.31-1.47]	7.52*** [6.93-8.17]
Cao Lanh district	1.27 (0.241)	2.16***	2.23** [1.25-3.96]	0.86 (0.511)		
Log(Density of veterinary drug shops)	0.79 (0.121)	1.12 (0.367)	1.58** [1.13-2.20]	0.85 (0.350)		
Log(Density of chickens)	1.08 (0.436)	1.39 (< 0.001)		0.85 (0.121)		
Disease (Yes/No)	–	4.28 (< 0.001)	1.80* [1.02-3.18]	–		
Mortality (Yes/No)		4.64 (< 0.001)				
AMU	–	–		–		0.90*** [0.86-0.94]

HR = Hazard rate Ratio; *p < 0.05; **p < 0.01; ***p < 0.001; ‡‡p = 0.061; †Model intercept: −1.320 (SE 0.218); ††Model intercept: −6.644 (SE 0.221); ††† Model intercept = −3.150 (SE 0.218).

since there were some flocks where all birds died (Fig. 2d). The most commonly reported clinical signs reported in flocks were, in decreasing order, malaise (weekly probability 0.20; 95% CI 0.19-0.23); diarrhoea (0.06; 95% CI 0.05-0.07); respiratory signs (0.05; 95% CI 0.04-0.06); sudden death (i.e. no prior sign of disease) (0.03; 0.02-0.03), CNS signs (0.01; 95% 0.006–0.014), and lameness (0.01; 95% CI 0.01-0.02) (Supplementary Material Figure S1). 'Other' disorders included lack of appetite, dehydration, and anaemia. These were reported with a combined probability of 0.09 (95% CI 0.08-0.11). There were differences in the timing of the different conditions: whereas malaise, sudden death, diarrhoea were more often reported in the earlier period, respiratory signs were reported most commonly in weeks 7-13. The weekly incidence of mortality conditional to the presence of respiratory signs was 2.69 (1.27–4.11) for the first 1 to 4 week period, and 9.34 (95% CI 6.15–12.50) for the period spanning from 5 weeks to sale. For weeks reporting diarrhea, the weekly incidence of mortality increased from 4.87 (95% CI 2.91–6.84) (1 to 4 week period) to 13.7 (95% CI 8.98–18.40) (late period).

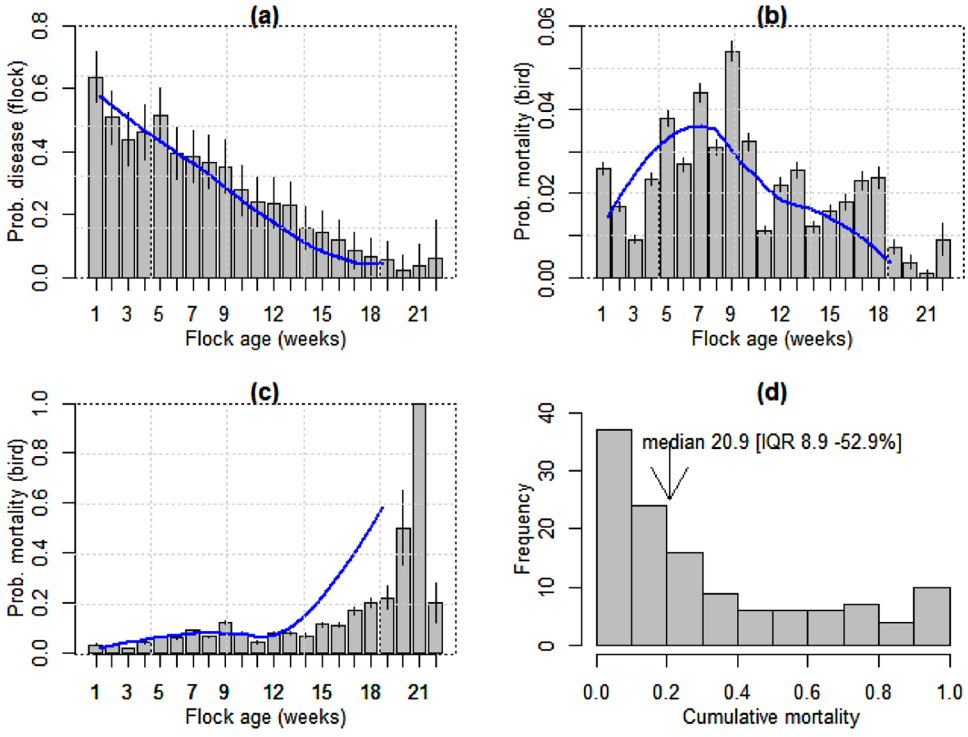

Fig. 2. (a) Probability of disease in flocks as a function of their age; (b) Overall weekly incidence of mortality over the observation period; (c) Probability of a bird dying conditional to being in a flock experiencing disease; (d) Frequency distribution of flock cycle (cumulative) incidence of mortality among 124 study flock cycles. The blue lines correspond to a smoothing function fitted by loess regression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

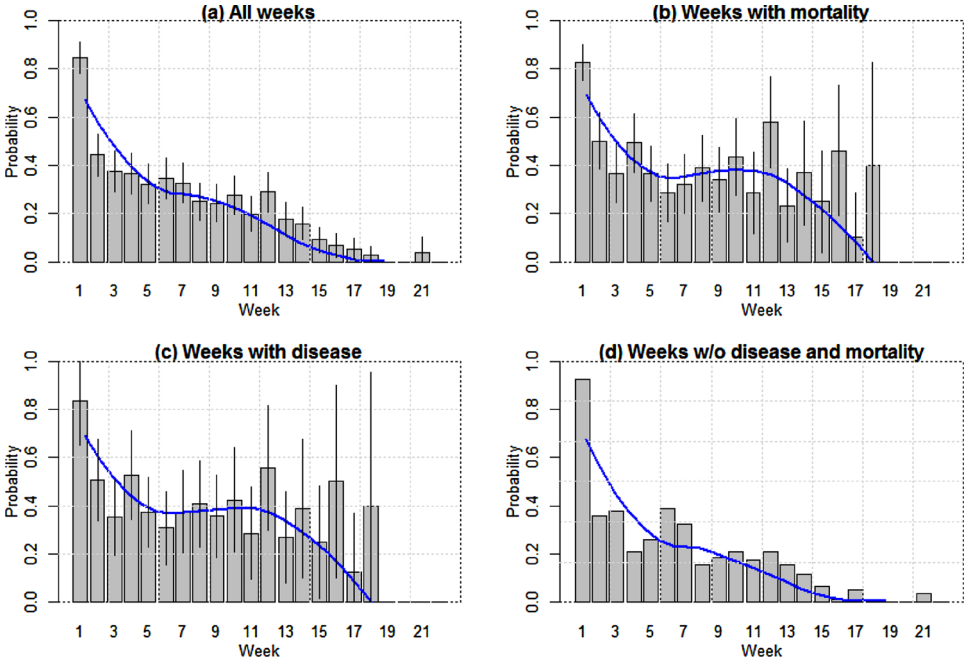

Fig. 3. (a) Overall probability of AMU by week; (b) Probability of AMU in weeks with mortality; (c) Probability of AMU in weeks with disease; (d) Probability of AMU in weeks without either disease and mortality (d). The blue lines correspond to a smoothing function fitted by loess regression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.3. Use of antimicrobials, vaccines and other health-supporting products

The five most common antimicrobials administered to flocks were colistin (13.9% observation weeks), followed by oxytetracycline (11.4%), tylosin (5.4%), doxycycline (4.4%), and gentamicin (3.0%) (data not shown). Flocks were vaccinated against a median of four different pathogens [IQR 3–4], the most common being Newcastle Disease (91.2% flocks), Highly Pathogenic Avian Influenza (82.4%), Infectious Bursal Disease (Gumboro) (80.0%), Fowlpox (43.2%) and Avian Pasteurellosis (28.0%). The impact of vaccination on disease was not investigated, since vaccines were applied at different times and data on timing of the application was missing in some cases. However, initial analyses did not reveal a significant association between vaccination against specific diseases (Yes/No) and the probability of disease (data not shown). In addition, other non-antimicrobial health-supporting products were used by farmers. These included vitamins/mineral complexes (93.6% flocks), digestive enzymes (77.7%), antiviral products (including interferon) (71.3%), mineral supplements (68.8%), coccidiostats (67.8%), electrolytes (49.5%), and anthelmintics (35.1%). The crude probability of AMU in a given week was 0.26 (95% CI 0.24-0.28) (Fig. 3a). This probability was inversely correlated with the age of the flock ($r = -0.89$; $p < 0.001$). In weeks when disease was reported, the probability of antimicrobial use was 0.43 (95% CI 0.41–0.48) (Fig. 3b, 3c), and 0.18 (95% CI 0.16–0.20) in weeks without disease. There was no difference in the probability of AMU depending on the reported clinical sign (range from 0.43 to 0.49 by clinical sign) (data not shown). There was a weak significant correlation between the probability of AMU in weeks with and without disease in the same flocks (Pearson's correlation = 0.391, $p < 0.001$) (Supplementary Figure S2). The probability of antimicrobials being used in weeks over subsequent cycles showed moderate correlation (Pearson's correlation = 0.459, $p < 0.001$). This observed correlation was greater than that correlation between the proportion of weeks with disease and/or mortality (Pearson's correlation = 0.040, $p = 0.8$) or average weekly incidence of mortality over two consecutive cycles (Pearson's correlation = 0.108, $p = 0.5$). There were marked differences in the probability of use of antimicrobials between the two study districts (Supplementary Figure S3).

3.4. Models of disease, mortality and antimicrobial use

Only the week of production was associated with 'Disease' (protective after 5 weeks) (OR ≤ 0.51). Factors independently associated with 'AMU' were: (1) No. of chickens (log) (OR = 1.46), (2) Stage of production ≥5 weeks (OR ≤ 0.67) (protective), (3) Cao Lanh district (OR = 2.64), (4) Density of veterinary drug shops at commune level (log) (OR = 1.58), and (5) Disease (OR = 1.80). The variable 'Density of veterinary drug shops at commune level', which was not significant in the univariable model, became significant after adjusting by district. Conversely, the variable Density of chickens became non-significant when the variable 'District' was added to the model. The variable Mortality become not significant when the variable Disease was introduced. The ICC associated with farm was for models explaining disease/mortality and AMU were 0.288 and 0.226, respectively.

Factors independently associated with overall increased weekly incidence of mortality ($p < 0.05$) were: (1) High level of education attainment (secondary education or higher) (Hazard rate Ratio [HR] = 1.70), (2) Number of chickens (log) (HR ≥ 1.39), and (3) Stage of production > 5 weeks (HR ≤ 2.14). In the model using the subset of weeks where farmer reported disease (N = 679) with weekly incidence of mortality as the response variable, all three variables fitted in the overall weekly incidence of mortality model remained significant: (1) High level of education attainment (secondary education or higher) (HR = 1.58), (2) Number of chickens (log) (protective) (HR = 0.89), and (3) Stage of production (HR ≥ 2.87). In addition, AMU remained as a significant (protective) factor (HR = 0.90). The two study districts differed in the percent of female farmers: 21.7% (10/46) in Thap Muoi vs. 2.4% (1/42) in Cao Lanh (Fisher's test, p = 0.08). The density of veterinary drug shops by commune in Cao Lanh was also higher than in Thap Muoi (3.1 vs. 1.80 per 10 sq. km) (Kruskal-Wallis $\chi^2 = 3.46$; p = 0.062). Also, the density of chickens in Cao Lanh communes was greater than in Thap Muoi (595.2 vs. 190.6 chickens per km^2, respectively (Kruskal-Wallis $\chi^2 = 2.76$; p = 0.039). Unlike the variables 'Density of chickens' and 'Density of veterinary drug shops', the variable 'Female' did not remain significant in the AMU model, suggesting that other unmeasured district-associated factors may account for the observed differences.

4. Discussion

We characterized disease, mortality, and AMU in small-scale chicken flocks in the Mekong Delta of Vietnam. Although highly variable across all production cycles, the average weekly incidence of mortality was 2.6% (equivalent to a monthly mortality of ˜11%), and the average flock cycle incidence of mortality was ˜33%. We believe that the data collected in this study reflect 'typical' farming practices, given that farmers did not receive any advice on husbandry/management practices from the research team. A major limitation of the study lies in the fact that disease status was assessed by farmers, introducing an element of subjectivity, since for some farmers some clinical signs may have appear to be 'normal' but not for others, based on their knowledge and experience. In addition, the data on flock disease, mortality and AMU was collected weekly, rather than daily. This did not allow determining in some cases whether the use of antimicrobials precluded the disease onset (prophylactic) or occurred in response to disease (i.e. therapeutic). We believe that, however, the data as a whole represents a valuable source of information on disease and mortality in these small-scale farming systems.

The observed high losses represent a major constraint to the productivity of small-scale systems. This magnitude was considerably higher than that reported from other studies from southern Asia. For example, a study on rural backyard chicken flocks in Cambodia reported average monthly mortalities of 4.5–6.3% (Conan et al., 2013), and a study on scavenging flocks in Bangladesh reported a 2.5% monthly mortality attributable to infectious disease (Biswas et al., 2006). However, in the latter study an additional 2.3% (monthly) mortality due to predation was reported. All our study flocks were penned and often fenced/protected by a mesh during the early brooding period, yet in a few cases chicks were predated by rats in the first few days of life (data not shown). A study from Nigeria reported an average cumulative mortality of 10.4% in small-scale poultry flocks (Muhammad et al., 2010). Our results also indicate a two to three times higher weekly incidence of mortality in these small, commercial farming systems, than in small backyard (median 16 birds [IQR 10–40]) flocks in the Mekong Delta of Vietnam (˜0.75 birds per week) (Delabouglise et al., 2018). There are no comparable data with slow-growing meat chicken flocks. Our observed flock incidence of mortality (˜33%) was also considerably higher than in broiler flocks in Nigeria (12%) (Odemero and Oghenesuvwe, 2016), Norway (2.9% excluding outliers) (Heier et al., 2002) and France (2.7%) (Chauvin et al., 2011).

The probability of disease was highest during the first period of the life of the flock, gradually decreasing thereafter. In contrast, mortality reached a peak during the central 5 to 10-week period, coinciding with the first phase of the 'grow-out' period, when chicks are allowed to access to a larger surface of the chicken house, often involving significant changes in feed type and litter conditions. A number of reasons may explain this: (1) waning of maternal and/or vaccinal protective antibodies; (2) increased pathogen challenge in the new environment; and (3) reduced attention paid to the flock by the farmer. Interestingly, it was during the mid-period, when respiratory problems were more often reported. Since diagnostic tests were not performed in our study, it is possible to determine the pathogens responsible for this. Pathogens such as Newcastle Disease virus, HPAI, Infectious Laringotracheitis (ILT) and IBV followed by secondary bacterial infections, or fowl cholera may account for some of this mortality. In addition to HPAI, there is the certainty that Newcastle Disease virus (Choi et al., 2014), and Infectious Bronchitis virus (IBV) (de Witt et al., 2010) are widely circulating in the area (Bui et al., 2001).

Our study confirmed that the presence of disease, rather than mortality, was a key explanatory factor for AMU in small-scale chicken flocks. Older flocks were less likely to be medicated, regardless of the presence of disease. The practice of using antimicrobials to prevent (rather than to treat) disease has been reported previously in chicken farms in the region (Carrique-Mas et al., 2014). Overall, the timing of AMU overlapped well with the presence of disease on farms. In Vietnam a large number of products are marketed as 'brooding medicine' ('thuôc um'), which almost invariably include one or several antimicrobial active ingredients. These products are often supplied by traders together the purchased day-old chicks as a 'package' (See Supplementary Figure S4 for a description of four representative products). Day-old chicks are typically brought to the farm by traders on motorbike, often involving travelling for over 100 km under a hot and humid climate, often resulting in poor condition of birds on arrival. Hatchery sources have been found to be associated with mortality in a number of studies (Heier et al., 2002; Muhammad et al., 2010). The data clearly showed that farmers tend to repeat their antimicrobial use patterns over subsequent cycles. Surprisingly, we found that in about ˜50% of weeks where flocks had overt signs of disease farmers did not administer antimicrobials. This occurred in situations when farmers judged the disease episode as mild, or in situations when farmers administered non-antimicrobial medicinal products such as vitamin complexes, minerals, enzymes, antibodies, and interferon (against suspected viral infections). We found that larger flocks had generally increased mortality and increased AMU levels. This contrasts with previous findings from a survey of poultry farms in a different province in the Mekong Delta, where smaller farms were at increased risk of AMU (Carrique-Mas et al., 2014). However, in that study smaller flocks were mostly backyard flocks, whereas all our study flocks were confined and single age. Interestingly, the density of veterinary drug shops was positively associated with increased AMU (OR = 1.58), suggesting that the availability of antimicrobials in veterinary drug shops may be a driving factor for AMU. In a previous study in another province in the Mekong Delta the veterinary drug shop was cited by 56% chicken farmers as their main source of procurement and advice of antimicrobial drugs to the farmers (Carrique-Mas et al., 2014). The differences observed between districts may also respond to differences in purchasing power of farmers these two districts. In addition, Cao Lanh district is closer to the provincial capital, with many more veterinary drug shops within close range. We have no explanation for the higher levels of mortality in flocks owned by farmers with higher education attainment. We did not find that this association was confounded by experience, district or any other variable. A possible explanation for this is that education is a proxy of wealth, and wealthier farmers have a wider range of occupations, and may therefore be less committed to tending their flocks. Given the presence of disease in the flock, the use of antimicrobials resulted in significantly lower weekly incidence of mortality (HR = 0.90), suggesting that therapeutic use of antimicrobials somehow reduces losses due to disease, although the magnitude of the observed reduction is small.

Our study focused on non-intensive, commercial chicken farms. Non-industrial farming production still account for the majority (60%) of chicken production in Vietnam (65% in the Mekong Delta region) (VCNST, 2018). The fragmentation of the Vietnamese farming landscape and the country's dependence on imported animal feeds, represent a major constraint to large-scale industrialization of poultry production (Ipsos Business Consulting, 2018). In addition, the Vietnamese consumer has a predilection for traditional, slow-growing breeds due to improved taste and texture. However the prolonged raising period required for these breeds represents an additional risk of disease introduction (Hong Hanh et al., 2007a).

5. Conclusions

We report exceptionally high levels of mortality in small-scale chicken flocks based on slow-growing breeds, and a clear association between the early brooding phase and the presence of disease and/or mortality and AMU in flocks. In addition, the link between AMU and the density of veterinary drug shops at commune level, as well as other unidentified district-related factors, suggest that the market availability of antimicrobials and other cultural factors may contribute to explain

AMU on farms. The study also highlights the benefits of regular (ideally daily) data collection on disease and mortality at farm-level, and therefore we encourage producers in the area to follow this practice. The results strongly suggest that farmers need to focus their efforts on controlling disease and mortality during the first 10 weeks of the life of the flock, improving chicken house sanitation and stepping up biosecurity to reduce the risk of introduction of disease. The presence of large numbers of small-scale chicken farms presents major challenges to the development of policies aimed at AMU reductions. We recommend that these policies include the stewardship of the antimicrobial use in farming systems in the region.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2019.02.005.

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Assessing antimicrobial misuse in small-scale chicken farms in Vietnam from an observational study

Marc Choisy[1,2,3,10*], Nguyen Van Cuong[1], Truong Dinh Bao[1,3], Bach Tuan Kiet[4], Bo Ve Hien[4], Ho Viet Thu[5], Niwat Chansiripornchai[6], Erry Setyawan[7], Guy Thwaites[1,8], Jonathan Rushton[9] and Juan Carrique-Mas[1,8]

Abstract

Background: Antimicrobials are used by poultry farmers in Vietnam as a tool to treat and prevent infectious diseases. We aimed to determine the fraction of disease episodes likely to remain untreated due to the administration of antimicrobials on non-susceptible pathogens in chicken flocks in the Mekong Delta of Vietnam. Weekly data on antimicrobial use and clinical signs were collected from 88 randomly chosen chicken flocks over 124 full production cycles (i.e. time between restocking flocks with day-old chicks and sale for slaughter). A naïve Bayes model was trained to infer the probabilities of disease episodes having been caused by each of 24 pathogens, given the observed clinical sign profile, and expert knowledge on their relative incidence.

Results: A total of 224 disease episodes were observed, of which 44.8% were attributed to viruses (95% CI 31.1–58.4%), 54.6% (CI 40.4–68.7%) to bacteria, and 0.6% (CI 0–1.7%) to a protozoan (*Eimeria* spp.). Antimicrobials were more frequently administered on weeks with disease than on weeks without disease (43.3% vs. 17.8%; $p < 0.001$). A median of 2 [IQR 0–4] antimicrobials were used by episode. The choice of specific antimicrobials was independent on whether the flocks had disease clinical signs or not. Antimicrobials were not used in 30.3% of the episodes. The overall probability that episodes were not effectively treated was 74.2, and 53.7% when discounting cases where the inferred aetiology is viral. Considering only episodes where antimicrobials were given, these probabilities were 57.4 and 23.8% respectively.

Conclusions: This study highlights untargeted use of antimicrobials on small-scale Vietnamese chicken farms, as well as the limitations of antimicrobials as effective tools to control infectious diseases.

Keywords: Antimicrobial usage, Chicken farm, Low- and middle-income country, naïve Bayes model

Background

Resistance against antimicrobials ("antimicrobial resistance", AMR) in animal production has received a great deal of attention in recent times, particularly given its serious implications for human health [1–3]. Zoonotic transmission of resistant organisms or AMR-encoding genes may result from environmental exposure of humans to livestock or its excreta, and/or from the transmission of livestock-borne resistant bacteria/genes through the food chain [4]. Antimicrobials are useful tools to control infectious diseases in animal populations [5]. Recently a consensus has built around the need to restrict their use other than for strict therapeutic purposes, in order to limit the emergence of antimicrobial resistant bacteria [6]. AMR in bacterial pathogens is hypothesized to reduce the effectiveness of antimicrobials in livestock production systems leading to lower levels of profitability and productivity of these systems [7].

With over 100 million tons of meat produced per year (2014), chicken represents the second most common animal food commodity worldwide [8]. Antimicrobials are extensively used in poultry farming, especially in low- and middle-income countries (LMICs) [9]. Faced with an episode of disease in the flock, the administration of

* Correspondence: marc.choisy@ird.fr
[1]Oxford University Clinical Research Unit, Hanoi, Vietnam
[2]MIVEGEC, IRD, CNRS, University of Montpellier, Montpellier, France
Full list of author information is available at the end of the article

antimicrobials is likely to be ineffective when there is mismatch between the chosen antimicrobials and the causative pathogens. This is expected when antimicrobials are administered to treat diseases caused by (1) a non-target organism (i.e. a virus, a fungus, or an intrinsically resistant parasite or bacterial strain), or (2) bacterial organisms that have acquired resistance to the antimicrobial. This is likely to be a common situation where the aetiological agent is not adequately diagnosed, and the choice of antimicrobial is not based on diagnostic or antimicrobial susceptibility testing results. Since veterinary diagnostics are normally not available to small-scale farmers typical of many developing countries, the antimicrobial susceptibility patterns of bacterial organisms is unknown, and choice of antimicrobials is mostly determined by their availability and cost.

Here we develop an original naïve Bayes model approach integrating clinical signs and weekly antimicrobial use (AMU) data from 124 chicken production cycles in 88 small-scale farms of the Mekong delta, Vietnam, allowing to estimate to what extent disease episodes are not effectively treated. Ineffective treatments are expected to fail to treat the disease, leading to a cost due not only to the treatment itself, but also to the loss of production. Ineffective treatments are also likely to contribute to increase the level of resistance in both commensal and pathogenic bacteria. Our method makes full use of available information from the literature and expert opinion in view of the considerable information gaps on diagnostics and antimicrobial sensitivity test (AST), which is often the case in LMICs. These are also the countries that bear the greatest burden of AMR infections [10]. There is unfortunately no way to validate our method. However, since the whole approach is entirely probabilistic, we were able to quantify and accumulate sources of uncertainty along the different steps of the analysis, building confidence intervals around our final estimates. Thus, if not perfect, this method has the advantage of being affordable whilst providing estimates that take into account any uncertainties about the data. Our method may not be useful to improve the situation of a particular farm but it is likely to be of invaluable use in giving recommendations for a local geographical level (district of province).

Methods

Farm selection and on-farm data collection

Eighty-eight (88) small-scale farms raising meat chicken flocks were randomly selected from the official census held by the veterinary authorities of Dong Thap province (Mekong Delta, Vietnam) (Sub-Department of Animal Health and Production, SDAHP) in the Cao Lanh and Thap Muoi districts, as part of the "baseline" (observational) phase of a research project [11]. All study farms restocked with 100–2000 chickens for each cycle of production. The chickens are predominantly of native breeds, with a growing period of 3–5 months. The farmers typically sell their birds to local markets with limited household consumption, and their inputs, including day-old-chicks, are also sourced locally. Farmers were provided with a structured diary and were instructed to weekly record information including: (1) clinical signs of disease in the flock: malaise (i.e. prostration, unwillingness to move, ruffled feathers), respiratory distress (sneezing, coughing, nasal/ocular discharge, difficult breathing), diarrhoea (watery faeces), alterations of the central nervous system (CNS) (ataxia, circling, torticollis), leg lesions, sudden death (i.e. death with no clinical signs); and (2) use of antimicrobial drugs (commercial products). Farmers were trained by SDAHP veterinarians on recognition of the six above-listed clinical signs, and were asked to keep containers of all antimicrobial products used. For each production cycle, farms were visited four times, during which records in the farm's diary were checked, and labels of antimicrobial products used reviewed. Individual antimicrobial active ingredients were entered into a dedicated database through a web application. All visits and data entry were carried out by trained veterinarians affiliated to the SDAHP.

Expert opinion on disease frequency

Three veterinarians based in Southeast Asia with experience in poultry medicine were independently asked to score the frequencies of 25 common chicken infectious diseases in the region. These pathogens included 14 bacteria: *Listeria monocytog*enes, *Avibacterium paragallinarum*, *Chlamydia psittaci*, *Clostridium perfringens*, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Gallibacterium anatis*, *Mycoplasma gallisepticum*, *Ornithobacterium rhinotracheale*, *Pasteurella multocida* (acute and chronic infections), *Pseudomonas* spp., *Salmonella* Gallinarum, *Salmonella* Pullorum, *Staphylococcus aureus*; 9 viruses: Avian Encephalomielitis virus, Highly Pathogenic Avian Influenza (HPAI) virus, Avian Metapneumovirus, Chicken Anaemia virus, Infectious Bursal disease (Gumboro) virus, Infectious Bronchitis virus, Infectious Laryngotracheitis virus, Marek's disease virus, Newcastle disease virus; and 1 protozoarian parasite (*Eimeria* spp.). The scores of each expert were then scaled to sum up 100, in order to produce values of relative frequency and the average of these 3 scorings was considered in the analysis. Because we distinguished between the acute and chronic infections caused by *Pasteurella multocida*, we will refer to 25 "pathogens" instead of 24 in the rest of the article.

Aetiology and antimicrobial resistance data from the literature

We reviewed standard veterinary textbooks on avian diseases [12, 13] to compile a presence/absence matrix of the 6 above-listed clinical signs caused by the 25 above-listed

pathogens. We added to this matrix age information, i.e. whether the pathogens are reported for young (< 7 week-old) and old (> 6 week-old) individuals, producing a final "aetiology" matrix of 25 (pathogens) rows and 6 (clinical signs) plus 2 (young and old) columns (Fig. 1).

We used a recently published literature review on the resistance of bacterial pathogens against antimicrobials [14] to produce a "resistance" matrix of 25 (pathogens) rows and n (drugs) where n was the total number of drugs recorded during the study, see Fig. 2. Each cell of this matrix contains values between 0 (fully susceptible) and 1 (fully resistant), quantifying the resistance of a pathogen to an antimicrobial drug. Missing values from a given drug/pathogen combination were imputed from the mean of the values for the drugs of the same class and the same pathogen when possible. When imputation was not possible (because absence of data on all the drugs of one class), we considered the mean of the values given by the three independent veterinarian experts.

Analysis

A "disease episode", defined as a succession of weeks during which clinical signs were reported, was considered out unit of analysis. In order to account for deficiencies in detecting/reporting clinical signs on farm, we assumed that single weeks without clinical signs but preceded and followed by weeks where clinical signs were reported were all part of the same disease episode. A disease episode was then characterized by the set of clinical signs observed and the set of antimicrobials administered during any week of the episode.

The analysis was then developed in two stages. The first one consisted in inferring the aetiologies of disease episodes from their sets of clinical signs, as well as the aetiology matrix and the expert opinion data, using a naive Bayes model framework [15]. The aetiology matrix was used to train the model, and expert opinion data was used as prior information. Note that here, in absence of diagnostic tests, the training phase did not include any validation step. The aetiology matrix from the literature was the only source of information available to train the model. Once trained, the model was applied to the set of clinical signs of each disease episode in order to derive a vector of 25 probabilities (adding up to 1), each probability of that vector quantifying the relative chance that the disease episode was caused by a particular pathogen. We used a Laplace correction factor of 1 in order to account for the fact that observed combinations of clinical signs may not perfectly match any of the combinations of the aetiology matrix. The successive steps leading to the inference of aetiologies of disease episodes are sketched in Fig. 1.

In the second stage of the analysis, for each disease episode, the above-derived aetiology probabilities were then used together with the set of antimicrobials used during the episode and the resistance matrix in order to derive the probability that the applied treatment was ineffective for treating the disease. For that, the resistance matrix was subsetted by column for the drugs used

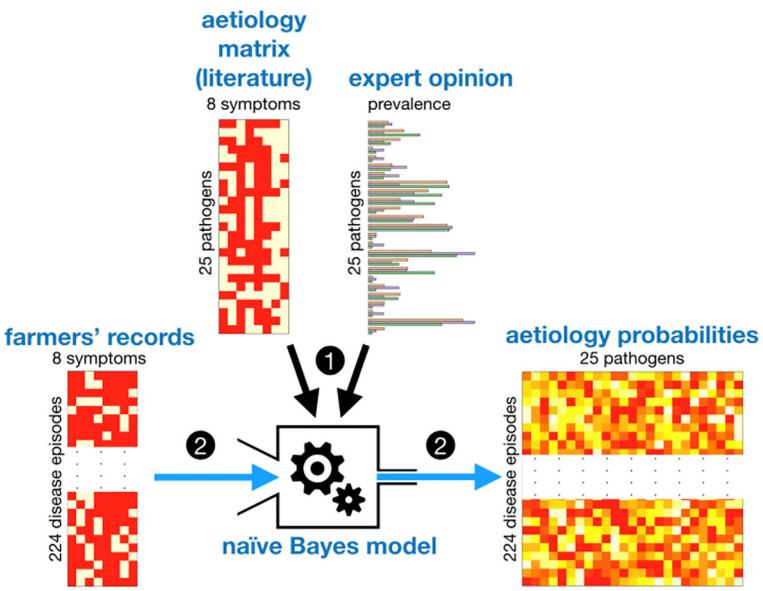

Fig. 1 Inferring the aetiologies of diseases episodes. (1) A 25 × 8 presence/absence matrix of clinical signs (and age of infection) per pathogen and the average relative prevalence scores from 3 independent veterinarian experts (top) are used to train a naive Bayes model (centre). (2) The naive Bayes model is then used to convert, for each disease episode, clinical signs and age surveillance data (presence/absence, left) into a vector of aetiology probabilities (right)

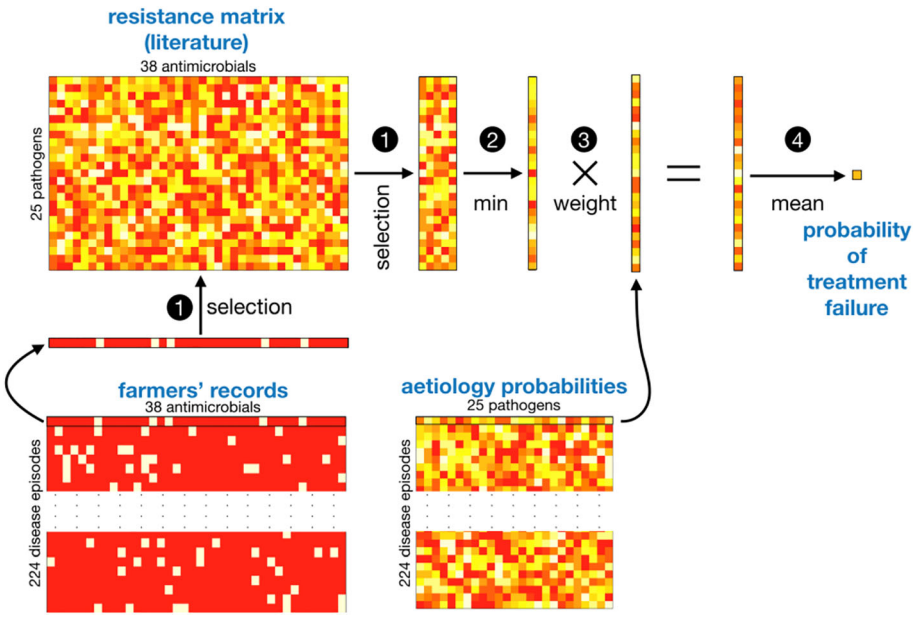

Fig. 2 Computing the probability of treatment failure of disease episodes. (1) For each disease episode, we subset the resistance matrix with the drugs used during the disease episode. (2) Then, for each pathogen of the subsetted matrix, we select the minimal level (min) of resistance across the used drugs. (3) The resulting vector is weighted (element-wise multiplication) by the vector of aetiology probabilities computed for that disease episode by the naive Bayes model (Fig. 1); and, (4) The mean of that weighted vector is used as the probability of treatment failure in the disease episode

during the disease episode. The minimal values by row (i.e. for a given pathogen) were then computed, producing a vector column of 25 values for the 25 pathogens. The values of this vector were weighted (element-wise multiplications) by the values of the vector of aetiology probabilities and then averaged, producing a probability that the used antimicrobials are ineffective in treating the disease. The successive steps leading to inference of this probability are sketched in Fig. 2.

Results

Farms, production cycles and disease episodes

The 88 farms were followed to include a total of 124 full production cycles (54 over one cycle, 32 over 2 cycles; 2 over 3 consecutive cycles). A total of 224 disease episodes were observed over all cycles. The median duration of one cycle of production was 18 [IQR 17–20] weeks. Clinical signs were recorded in 116/124 (93.5%) cycles of production. The median duration of disease episodes was 2 [IQR 1–4] weeks. Disease episodes spanned a median of 22.7% [IQR 10.0–40.0] observation weeks. The most common clinical signs reported were, in decreasing order, malaise (81.2% episodes), diarrhoea (29.0%), respiratory distress (24.1%), sudden death (15.2%), leg lesions (11.1%), and alteration of the CNS (0.8%). The probability of disease markedly decreased with the age of the flock (Fig. 3).

Inference of aetiological agents from observed clinical signs

The most common types of clinical signs of the 25 poultry etiologic agents ("aetiology matrix") are presented in Additional file 1: Table S1. There was reasonable agreement between all three reviewers in their scoring of disease by their relative frequency (r values between 0.78 and 0.89) (Additional file 1: Fig. S1).

Results from the naïve Bayes model expressed as relative probability (by episode and by cycle of production) are presented in Table 1. There was a very strong correlation between the relative probability of each pathogen expressed by week and by episode ($r = 0.954$; $p < 0.001$). The model attributed 44.8% (95% CI 31.1–58.4%) episodes to viral pathogens, 54.6% (95% CI 40.4–68.7%) to bacterial pathogens, and 0.6% (95%CI 0–1.7%) to *Eimeria* spp. (Table 2). The bacterial infections most commonly predicted were, in decreasing order: (1) *Erysipelothrix rhusiopathiae* (probability per episode 0.079); (2) *Gallibacterium anatis* (0.073); (3) *Mycoplasma gallisepticum* (0.068); (4) *Salmonella* Pullorum (0.068), and *S.* Gallinarum (0.043). The most commonly predicted viral infections were, in decreasing order: (1) Infectious Bursal disease (IBD) (0.162); (2) Avian Metapneumovirus infection (0.105); (3) Marek's disease (0.057); (4) Infectious Laryngotracheitis (0.038); and (5) Newcastle disease (0.034) (Table 1). There was a strong positive correlation between the probability attributed to a

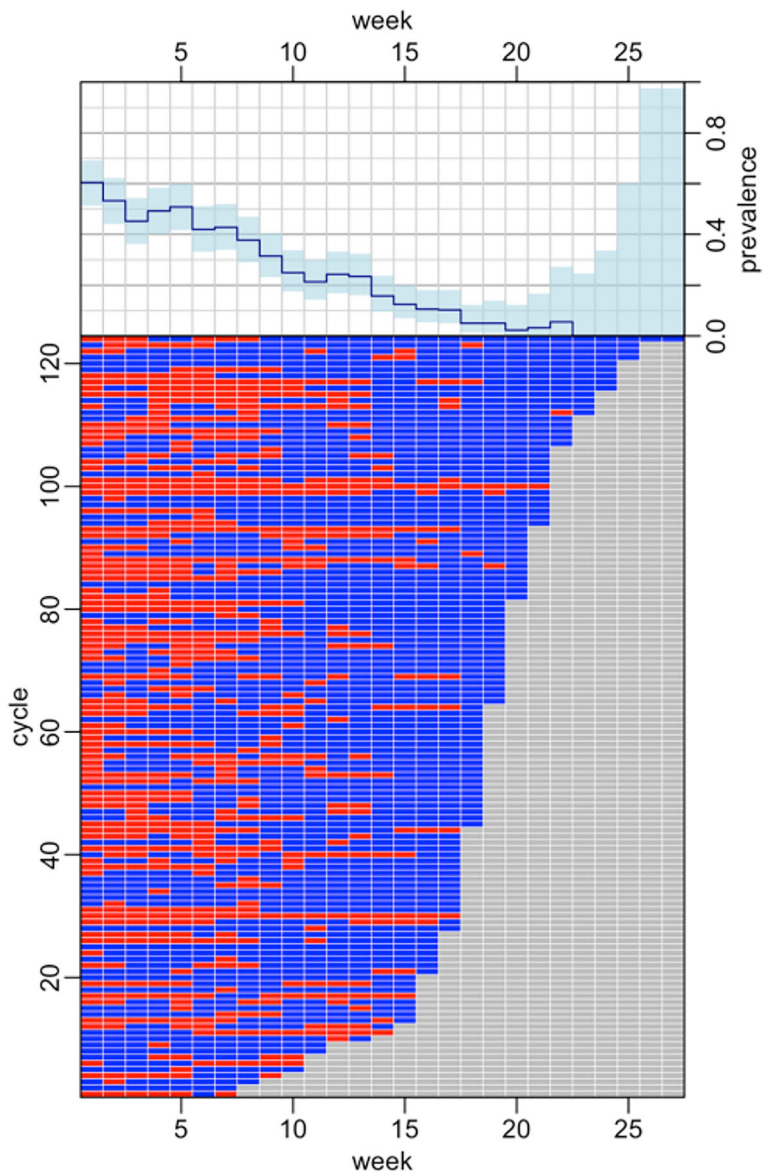

Fig. 3 Disease episodes of over the 124 full cycles of production. Top: prevalence of clinical signs of disease on farms (with 95% confidence interval), by week. Bottom: production weeks with (red) and without (blue) disease episode

bacterial pathogen and the duration of episodes ($r = 0.37$; $p < 0.001$).

Overall, there was a reasonable agreement between the prior probabilities estimated by the naïve Bayes model and the average of the three poultry veterinary experts. However, the assessments of the experts on HPAI, *E. coli*, Infectious Bronchitis virus, *Avibacterium paragallinarum* and *Eimeria* spp. are higher than the incidences predicted by the model (Fig. 4). Conversely, their assessments on Avian Metapneumovirus, *G. anatis*, *E. rhusiopathiae*, and *Clamydia psittaci* are lower than the incidences predicted by the model (Fig. 4).

Antimicrobial use and disease episodes

Antimicrobials were more frequently administered on disease episode weeks (296/683, 43.3%), than in weeks without disease (281/1582, 17.8%) ($\chi^2 = 163.0$, $p = 0.001$) . Similar to the probability of disease, the weekly probability of antimicrobial usage decreased with the age of the flock: from 0.84 (week 1), to 0.31–0.44 (weeks 2–7), 0.10–0.30 (weeks 8–15), and < 0.10 thereafter. Farmers did not use antimicrobials in 88/224 (39.3%) of disease episodes. Thirty-eight (38) different types of antimicrobials were used by farmers (Additional file 1: Table S2). The most frequently used antimicrobials were: colistin (12.2% weeks across farms), oxytetraycline (9.8%), tylosin

Table 1 Average probabilities (with 95% confidence intervals) of each of the pathogens (in row) to be the etiological cause of a disease episode or a disease episode in a cycle of production. Note that the probabilities do not necessarily sum to 1 by row because they are averages by episode and cycle of production. Note also that the probabilities averaged by episode can be compared to the mean of the score of the 3 independent experts

Pathogen	Episode			Cycle of production	
	Model	95% CI	Expert opinion	Model	95% CI
Infectious Bursal Disease virus	0.162	0.113–0.210	0.101	0.303	0.101–0.506
Avian Metapneumovirus	0.105	0.064–0.145	0.014	0.194	0.044–0.345
Erysipelothrix rhusiopathiae	0.079	0.044–0.115	0.009	0.147	0.044–0.251
Gallibacterium anatis	0.073	0.039–0.107	0.008	0.137	0.030–0.244
Mycoplasma gallisepticum	0.068	0.035–0.101	0.074	0.127	0.029–0.225
Salmonella Pullorum	0.068	0.035–0.101	0.042	0.127	0.042–0.213
Marek's Disease virus	0.057	0.026–0.087	0.059	0.105	0.000–0.219
Salmonella Gallinarum	0.043	0.016–0.069	0.028	0.080	0.028–0.133
Infectious Laringotracheitis virus	0.038	0.013–0.063	0.022	0.036	0.007–0.064
Clostridium perfringens (necrotic) enteritis)	0.038	0.013–0.063	0.059	0.071	0.004–0.138
Escherichia coli (colibacillosis)	0.034	0.011–0.058	0.106	0.063	0.023–0.102
Newcastle Disease virus	0.034	0.010–0.057	0.079	0.064	0.000–0.133
Chlamydia psittaci	0.034	0.010–0.057	0.006	0.063	0.016–0.111
Staphylococcus aureus	0.032	0.009–0.054	0.024	0.059	0.013–0.105
Chicken Anaemia virus	0.031	0.008–0.053	0.022	0.057	0.016–0.098
Pasteurella multocida (acute) (fowlcholera)	0.025	0.004–0.045	0.035	0.047	0.003–0.091
Ornithobacterium rhinotracheale	0.023	0.003–0.042	0.025	0.042	0.007–0.077
Listeria monocytogenes	0.012	0.000–0.026	0.009	0.022	0.000–0.043
Infectious Bronchitis virus	0.011	0.000–0.024	0.060	0.020	0.008–0.033
Avian Encephalomielitis virus	0.009	0.000–0.021	0.011	0.008	0.000–0.016
Avibacterium paragallinarum	0.008	0.000–0.020	0.031	0.015	0.000–0.032
Eimeria spp.	0.006	0.000–0.017	0.038	0.012	0.003–0.022
Pasteurella multocida (chronic)	0.005	0.000–0.015	0.011	0.010	0.000–0.020
Pseudomonas spp.	0.005	0.000–0.014	0.011	0.009	0.000–0.018
Highly Pathogenic Avian Influenza virus	0.002	0.000–0.008	0.113	0.004	0.000–0.011

(4.8%), and doxycycline (3.7%). These four antimicrobials represented 53.1% of total usage. In episodes where antimicrobials were used, the median number of different antimicrobials used was 3 [IQR 2–4]. There was no evidence that different antimicrobials are more likely to be used in situations of disease, compared with no disease (Fig. 5). Episodes where no antimicrobials were used had a shorter duration (median 1 [IQR 1–2] weeks) compared with episodes where antimicrobials were used (median 3 [IQR 1–5] weeks) (Wilcoxon test, $W = 3120$; $p < 0.001$).

Phenotypic resistance of bacterial organisms

The full list of antimicrobials used, alongside the prevalence of resistance of poultry pathogens against them is presented in Additional file 1: Table S2.

Probability that disease in flocks remains untreated

The overall probability (all episodes) that a disease episode remains untreated (either because of absence of treatment, or because of ineffective treatment) was 74.2% (95% CI 68.4–79.9%) for all episodes, and 53.7% (95% CI 47.2–60.3%) for episodes due to bacterial pathogens (including *Eimeria* spp.). For episodes where antimicrobials were given, the estimated treatment failure was 57.4 (51.0–63.9%) (all pathogens), and 23.8% (95% CI 18.2–29.4%) (bacterial pathogens). The probability of failing to treat the disease in episodes where antimicrobials were given was very variable, ranging from 0.423 (*Ornithobacterium rhinotracheale*) to 0.030 (*Pasteurella multocida*) (Table 2). For bacterial pathogen, this probability was strongly dependent on the number of antimicrobials used (Fig. 6).

Table 2 Average probabilities (with 95% confidence intervals) that a disease episode caused by a given bacteria (by row) remains untreated either because of absence of treatment or because of ineffective treatment (first two columns), or because of ineffective treatment only (last two columns). The probabilities in the last two columns are necessarily smaller than in the first two columns

Bacterial pathogen	Overall treatment failure		Treatment ineffective when antimicrobial/s given	
	Mean	95% CI	Mean	95% CI
Avibacterium paragallinarum	0.499	0.433–0.564	0.175	0.125–0.224
Chlamydia psittaci	0.554	0.488–0.619	0.265	0.207–0.322
Clostridium perfringens (necrotic enteritis)	0.595	0.530–0.659	0.332	0.271–0.394
Eimeria spp.	0.895	0.855–0.935	0.827	0.778–0.877
Erysipelothrix rhusiopathiae	0.611	0.547–0.675	0.360	0.297–0.422
Escherichia coli (colibacillosis)	0.555	0.490–0.620	0.266	0.209–0.324
Gallibacterium anatis	0.562	0.497–0.627	0.278	0.220–0.337
Listeria monocytogenes	0.602	0.538–0.666	0.344	0.282–0.406
Mycoplasma gallisepticum	0.645	0.582–0.707	0.415	0.350–0.479
Ornithobacterium rhinotracheale	0.650	0.587–0.712	0.423	0.359–0.488
Pasteurella multocida (*acute* (fowl cholera))	0.411	0.347–0.475	0.030	0.008–0.052
Pasteurella multocida (chronic)	0.411	0.347–0.475	0.030	0.008–0.052
Pseudomonas spp.	0.479	0.413–0.544	0.142	0.096–0.187
Salmonella Gallinarum	0.425	0.360–0.490	0.053	0.024–0.082
Salmonella Pullorum	0.425	0.360–0.490	0.053	0.024–0.082
Staphylococcus aureus	0.472	0.407–0.537	0.130	0.086–0.175
Overall (all episodes)	0.537	0.472–0.603	0.238	0.182–0.294

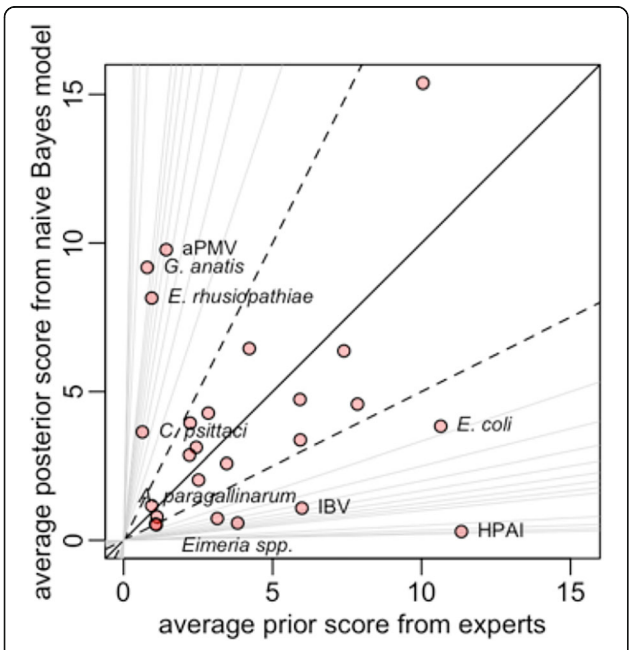

Fig. 4 Relationship between prior estimates from veterinary expert opinion and posterior probabilities predicted by the naïve Bayes model. Lines above the diagonal have slopes increasing from 2 (black dashed line), 3, … 10, 20, …50 (all grey lines). Similarly, lines below the diagonal have slopes decreasing from ½ (black dashed line), 1/3, … 1/10, 1/20, … 1/50 (all grey lines)

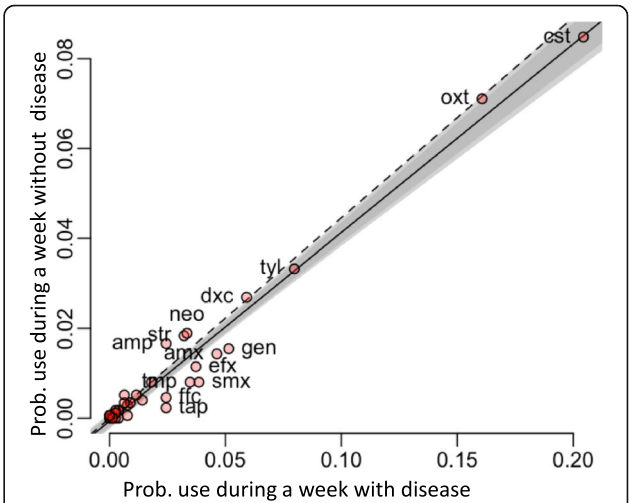

Fig. 5 Probability of use of antimicrobial active ingredients in weeks with and without disease. Relationship, for each antimicrobial, between the probability of use during a week with disease and without disease. The black line is the regression line and the light and grey areas are the 99 and 95% confidence intervals respectively. The dashed line is the expected relationship in case there is no difference of usage between the weeks with and without disease (the slope is equal to the ratio of weeks with and without disease). cst = colistin, oxt = oxytetracycline, tyl = tylosin, dxc = doxycycline, neo = neomycin, gen = gentamicin, str = streptomycin, amp = ampicillin, amx = amoxicillin, gen = gentamicin, efx = enrofloxacin, tmp = trimethoprim, smx = sulfamethoxazole, ffc = florfenicol, tmp = thiamphenicol

Discussion

Antimicrobials are formidable tools for the control of infectious diseases in animal production. The trade-offs of antimicrobial usage have been discussed, although focused on their costs vs. the benefits from protecting flocks/herds from disease [16]. This study is, to our knowledge, the first one to look into the likelihood of unsuccessful treatment of infectious diseases in small-scale farming systems in Asia, either because antimicrobials were not used, or because an ineffective antimicrobial were used. Key findings of this study are: (1) half (48.7%) antimicrobial use occurred on weeks without disease; (2) for episodes where antimicrobials were used, they were expected to be ineffective in 57.4% (CI 51.0–63.9%) episodes (for all pathogens considered), and 23.8% (18.2–29.4%) (for bacterial pathogens); (3) antimicrobials were not used in over a third (39%) of disease episodes.

Our analysis estimated that approximately a fourth (23.8%) of treated bacterial episodes are likely to be ineffective due to the organisms treated being resistant to the antimicrobials used. This outcome is likely a combination of "intrinsic" and "acquired" resistance properties of bacterial pathogens. However, in this paper we have not attempted to investigate the fraction likely due to acquired resistance since for many antimicrobials and pathogens this is now well known. Most published AMR data on poultry pathogens comes from studies in developed countries. Given the higher levels of antimicrobial use in Vietnamese chicken farms [17], it is likely that the resulting values of expected antimicrobial resistance are underestimated. We ignored the timing of application of the antimicrobial in relation to the onset of disease, or the order of the administration because this could not be determined from weekly data collection. Surprisingly however, in over a third of disease episodes (39%) farmers gave no antimicrobials at all, resulting in an even higher percent in overall failure to efficiently treat a bacterial disease episode (53.7%). When viruses are also considered, the overall fraction of treatment failure reached 74.2%, as ~ 45% of disease episodes were expected to be caused by viral pathogens.

Two assumptions of our study may have resulted in biased results. Firstly, the assumption that all disease episodes were either due to a bacterial or a viral pathogen, excluding helminth infections and other non-infectious aetiologies (i.e. toxicosis, metabolic disorders, etc.). However, given the farming conditions of small-scale farms in Vietnam, with generally serious deficiencies in biosecurity, it is likely that the overwhelming majority of over disease is infectious in nature. Secondly, the study is necessarily biased towards diseases that are easier to diagnose/detect. Interestingly the expert panel predicted HPAI and colibacillosis (*E. coli*) to be more common than what the model predicted after integrating data on clinical signs. Further diagnostic testing in the area by the authors has confirmed a lack of HPAI in the areas at the time of the study (data not shown). Surprisingly, the model and the experts predicted generally relatively low incidence of coccidiosis (*Eimeria* spp.), which is regarded as a major health problem in industrialised poultry production systems. It is believed that coccidial infectious are indeed present, but mostly the subclinical form is predominant, contributing to reduced intestinal functions [18]. Thirdly, we ignored data on vaccination (mostly to prevent viral infections) and assumed that the probability of an episode due to a given virus was not affected by whether the flock had been vaccinated or not. Farmers in the area apply vaccines notably against HPAI, IBD and Newcastle disease. However, the application of the vaccine requires careful logistics including adequate strain choice and logistics (timing, booster, storage and administration logistics) than more often than not were

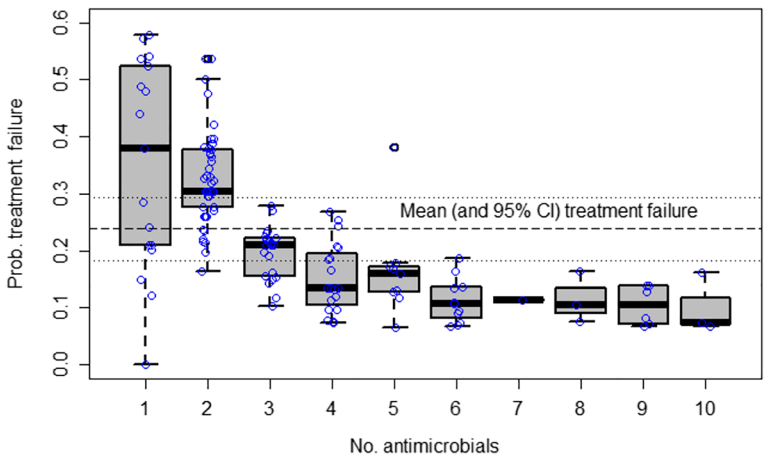

Fig. 6 Predicted summary treatment failure of individual episodes attributed to bacterial pathogens. The box indicates median values and 75% interquartile-range; whiskers indicate extreme values

not met. In the case of HPAI, there is some evidence that vaccination coverage is either low or application is performed poorly [19].

A third of disease episodes did not trigger farmers to administer antimicrobials. These episodes were typically short (one week) with non-specific signs of disease (i.e. malaise). Often in these cases, farmers used vitamins, probiotics, yeasts and antibodies to manage poultry health issues (data not shown). Interestingly, episodes attributed to bacteria tended to last longer, giving further empirical evidence to the phenomenon of AMR on farms.

Although most episodes were addressed by the administration of two antimicrobials, in some instances up to 10 different antimicrobial active principles were used by the farmer. This is not surprising, since many commercial antimicrobial formulations in the area include at least two antimicrobial active ingredients [20] and confirms high usage of antimicrobials in Vietnamese small-scale chicken farms [20, 21]. However, over 50% of total antimicrobial use corresponded to weeks with no disease reported (i.e. prophylactic use). This is likely to be partly triggered by fear of disease, either from previous experience or by the knowledge of presence of nearby disease, coupled with the lack of competent veterinary diagnostic/advisory capacity. As suggested in the introduction, there is a strong suspicion that the choice of antimicrobials is currently based on costs.

Some of the most commonly used antimicrobials (i.e. colistin, oxytetracycline) were associated with a high probability of ineffective treatment of the disease (data not shown). In the case of colistin, this reflects a high predicted incidence of *Gallibacterium anatis* infection (characterized by respiratory, diarrhoea and malaise, in all ages), and *Erysipelothrix rhusiopathiae* (malaise, sudden death, in all ages), both of which are often very resistant against these antimicrobials (≥40%). To the best of our knowledge, *Gallibacterium anatis* has never been isolated in Vietnam. Our results suggest that it could be valuable to include this pathogen in the diagnostic testing protocols. The use of colistin (and to lesser extent fluoroquinolones, macrolides, aminoglycosides and β-lactams), some of which are considered of critical importance for human medicine [22] is particularly worrying from a public health point of view.

Our approach is particularly useful in settings where diagnostic capacity (and AMR testing) is limited, such as many LMICs [23]. As more local epidemiological and microbiological data becomes available, through improved diagnostic and AMR testing, these can easily be integrated in our modelling framework to improve the precision and accuracy of our estimates. The approach can also help to focus diagnostic efforts towards those diseases that are considered more likely, as well as to review vaccination programmes. In generally, the model framework we developed here can be used for any system (animal or human) where clinical signs, antimicrobial use and AMR data are known to improve treatment success.

In summary, using a novel integrated methodology that combined data from expert opinion, literature and field observations, we investigated the relationship between AMU and infectious disease in smallholder poultry systems. When farmers used antimicrobials to address disease episodes in their flocks, failure to treat disease was expected in about ~ 57% cases (~ 24% assuming a bacterial causative agent). Our study shows a high frequency of usage of antimicrobials in situations with no disease, and absence of use when disease is present on flocks, the widespread use of multiple courses of different antimicrobials, and the random use of different antimicrobial products suggesting that there is ample room for improvement in the targeting of antimicrobials on farms in small-scale farming systems in Vietnam.

Conclusions

This study shows how clinical signs and antimicrobials usage surveillance data can be used to infer the level of antimicrobial misuse in chicken farms. The naïve Bayes framework that we employ allows to do so probabilistically, rigorously accounting for all sources of uncertainty. Our results show that a vast majority of disease episodes are likely to be not treated effectively, representing an important loss for the farmers. The method that we develop is general and can be applied to any set-up, including human infections. The model can also be used to improve the current treatments at use.

Additional file

Additional file 1: Figure S1: relationships and correlations between the scores of the 3 independent veterinarian experts on the frequencies of the 25 pathogens. **Table S1:** presence/absence aetiology matrix. "X" are used whenever a symptom (in column) has been reported for a given infection (in row) in standard veterinary textbooks on avian diseases (9, 10). **Table S2** Prevalence of resistance (in percentages) of the 16 poultry pathogens against the 39 antimicrobials considered in the study. Values come either from the literature (blue), expert opinion (green), or are inferred from the values of other antimicrobials in the same class (yellow). (DOCX 106 kb)

Abbreviations

AMR: Antimicrobial resistance; AMU: Antimicrobial use; AST: Antimicrobial sensitivity test; CI: Confidence interval; CNS: Central nervous system; HPAI: Highly pathogenic avian influenza; IBD: Infectious bursal disease; IQR: Interquartile range; LMIC: Low- and middle-income countries; OXTREC: Oxford tropical research ethics committee; SDAHP: Sub-department of animal health and production

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We are grateful to SDAHP staff and farmers for co-operation during data collection.

Authors' contributions

MC, BVH, GT, JR and JCM contributed to the conception and design of the study, NVC, TDB, BTK and ES were involved in the data collection, MC, TDB,

BTK, BVH, HVT, NC, ES, GT and JCM contributed to the analysis and interpretation of data, MC, NVC, JR and JCM drafted the manuscript and MC, NVC, TDB, BTK, HVT, NC, ES, GT, JR and JCM critically revised the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials
All the data sets used in this study as well as R code are available from https://doi.org/10.5281/zenodo.2611133, or https://github.com/viparc/treatfail for an up-to-date version.

Ethics approval and consent to participate
This is an observational study in which we collected disease and AMU related data from normal commercial farms. Before the onset of the study, all farmers were informed about aims and procedures of the study and were required to complete and sign consent form should they wish to join. This research has been carried out under the umbrella of the ViParc project (http://viparc.org), and has been granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Ref. 5121/16).

Consent for publication
Not applicable.

Competing interests
None of the authors has any competing interest.

Author details
[1]Oxford University Clinical Research Unit, Hanoi, Vietnam. [2]MIVEGEC, IRD, CNRS, University of Montpellier, Montpellier, France. [3]Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh city, Vietnam. [4]Sub-Department of Animal Health and Production (SDAHP), Cao Lanh, Dong Thap, Vietnam. [5]University of Can Tho, Can Tho, Vietnam. [6]Avian Health Research Unit, Chulalongkorn University, Bangkok, Thailand. [7]Food and Agriculture Organization of the United Nations, Jakarta, Indonesia. [8]Nuffield, Department of Medicine, Centre for Tropical Medicine and Global Health, Oxford University, Oxford, UK. [9]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK. [10]LMI "Drug Resistance in South-east Asia" (DRISA), Hanoi, Vietnam.

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Letter to the Editor

Affordability of antimicrobials for animals and humans in Vietnam: A call to revise pricing policies

Sir,

A recent review in the *International Journal of Antimicrobial Agents* revealed how the practice of purchasing antimicrobials over-the-counter and without a prescription is widespread in most low- and middle-income countries (LMICs) [1].

Antimicrobials for human medicine are commonly acquired without a prescription in Vietnam, despite legislation restricting this practice. Conversely, antimicrobials aimed at veterinary medicine can be legally purchased without prescription by anyone from any of the ~12 000 veterinary drug stores across the country. It is not known to what extent the ease of access and affordability contributes to excessive antimicrobial usage (AMU) in animal production. Furthermore, there is little information regarding the affordability of antimicrobials in different countries, or how their pricing compares with the equivalent antimicrobial drugs sold in human medicine.

As a component of a study of 270 cycles of production in 112 chicken farms in the Mekong Delta region of Vietnam [2], we identified 236 different products containing 42 different active antimicrobial ingredients. On average, five (interquartile range 2.25–10) antimicrobial products were used per flock cycle, which typically lasts for 16–18 weeks; the majority of AMU occurs during the early ('brooding') phase of production.

We calculated the cost incurred for the daily treatment of 1 kg of live chicken (equivalent of administering one animal daily dose kg, ADD_{kg}) for the 10 most common antimicrobial products used by farmers. The vast majority of products are powder based, and are administered orally after dilution with water. All products were purchased as 0.1-kg powder sachets. In calculating the dose, we followed the manufacturer's guidelines for their preparation and administration, assuming that a 1-kg chicken typically drinks 225 ml of water per day under local environmental conditions. We expressed the costs in cents of a US dollar (US$) (Table 1).

The price of 1 ADD_{kg} of antimicrobial product ranged from 0.19 to 1.03 US$ cents (average 0.56 US$ cents per kg of chicken). However, in many cases, the product labels include guidelines for prophylactic administration, requiring a lower (≤50%) concentration, and therefore representing less than half of the cost (i.e. on average <0.28 US$ cents per kg of chicken). The most commonly used product contained colistin, which was also the most affordable (0.19 and 0.07 US$ cents for therapeutic and prophylactic use, respectively). As a comparison, the equivalent costs of products containing the same antimicrobial sold for human use in Vietnam per kg recommended therapeutic dose (assuming a 60-kg weight for a human adult) were: thiamphenicol (1.61 US$ cents), gentamicin (0.87 US$ cents), streptomycin (0.78 US$ cents), doxycycline (0.55 US$ cents) and sulfamethoxazole (0.50 US$ cents). Vietnam is among the countries where AMU is expected to increase rapidly in the coming years [3]. It has been suggested that increasing user fees may deter excessive AMU in food animal production, and the increased revenues could be used to mitigate the consequences of antimicrobial resistance [4].

Assuming that a typical chicken is supplemented with antimicrobials for 40 of its 126-day life cycle), with an average weight at treatment of 0.25 kg, this would represent a cost of antimicrobials equivalent to ~0.03 US$ cents per chicken. Farmers in the Mekong Delta of Vietnam sell their slaughter-age chickens at 6 US$ per bird; thus, the cost of antimicrobials represents approximately 0.5% of the income raised from chicken sales. Although we do not have data on the price of antimicrobials in other animal species or in other LMICs, these prices seem to be remarkably low and are unlikely to be a limiting factor for unnecessary AMU. Directions for use indicating prophylactic dilution contribute to re-inforce the concept that the use of antimicrobials when the flock is healthy is appropriate. More often than not, antimicrobials are sold in combination with vitamins and other health-supporting substances. More worryingly, some of the most commonly used products in animals contain colistin, which is a critically important antimicrobial of the highest priority for human medicine.

Vietnam is an LMIC that does not manufacture active antimicrobial ingredients itself, instead relying on imports. These chemicals are mixed, packed and distributed within the country to meet local demand. We propose that an import tax on antimicrobials of critical importance for human use should be considered. With the exception of ampicillin, amoxicillin and their derivatives (subjected to 5% and 10% import tax, respectively), most antimicrobials are currently exempt of import tax in Vietnam [5]. In order to avoid these increases having a negative impact on the availability of antimicrobials of critical importance for human use when genuinely needed, we recommend effective enforcement of existing legislation to restrict over-the-counter access, while subsidising the use of these antimicrobials if acquired with a doctor's prescription.

An alternative would be to levy a tax for veterinary antimicrobial products. Anecdotal information from our interaction with farmers suggests that the majority would not alter their AMU behaviour substantially, even with a four-fold increase in the price of antimicrobials. Within the proposed tax system, antimicrobials of critical importance used in veterinary medicine should be allocated to the highest tax bracket. There is a risk that such increases may lead to the undesirable creation of a black market of cheap counterfeit products. However, as most farmers are not aware of the differential effectiveness or impact on antimicrobial resistance associated with the use of antimicrobials of critical importance, we believe this would likely result in a preferential choice of 'older-generation' types of antimicrobials. The revenues raised from this

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Table 1
The 10 most common antimicrobials used by a cohort of 112 farmers investigated over 270 cycles of production, and the prices of animal daily dose kg (ADD_{kg})

Product	Antimicrobial active principle	Volume (L) of antimicrobial solution prepared per sachet of product (prophylaxis/therapy)	No. of ADD_{kg} per sachet (prophylaxis/therapy)	Cost of 1 ADD_{kg} (range) (in US$ cents)	
				Prophylaxis	Therapeutic
1	Colistin + oxytetracycline	250/100	1111/444	0.07 (0.06–0.17)	0.19 (0.14–0.43)
2	Colistin + oxytetracycline	-/100	-/444	–	0.28 (0.10–0.48)
3	Colistin + gentamicin	-/50	-/222	–	0.44 (0.33–0.62)
4	Colistin + oxytetracycline	100/50	444/222	0.51 (0.29–0.58)	1.02 (0.58–1.16)
5	Oxytetracycline + streptomycin	-/50	-/222	–	0.42 (0.19–0.58)
6	Colistin + oxytetracycline	100/50	444/222	0.20 (0.15–0.43)	0.40 (0.29–0.97)
7	Sulphamethoxazole + thiamphenicol	67/33	296/148	0.51 (0.22–0.72)	1.03 (0.43–1.45)
8	Methenamine	100/67	444/296	0.53 (0.43–0.63)	0.79 (0.65–0.94)
9	Doxycycline + tylosin	400/200	1778/889	0.12 (0.04–0.16)	0.23 (0.07–0.31)
10	Gentamicin + tylosin	100/50	444/222	0.43 (0.14–0.58)	0.85 (0.29–1.15)

NI, not indicated.
Prices are expressed in US$ cents, based on an exchange rate of 1 US$=23 319 VND (23 September 2018)]. The products are sorted by frequency of use. All products were purchased as 100-g sachets.

tax could help train veterinary pharmacists to improve their prescription practices.

There is remarkable diversity in retail prices of antimicrobials for animal use [3] across LMICs, presumably reflecting differences in production capacity, market structure and AMU practices. As such, we propose that such a taxation system should be defined on a country-by-country basis. Crucially, the use of (any) antimicrobials as prophylactic agents should be discouraged in all cases, and veterinary drug manufacturers should make this explicit in the product labels.

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Competing interests

None declared.

Ethical approval

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Juan Carrique-Mas*
Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam
Centre for Tropical Medicine and Global Health, Oxford University, Oxford, UK

Nguyen Van Cuong, Bao Dinh Truong, Doan Hoang Phu, Tran My Phuc, Hugo Turner
Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Guy Thwaites, Stephen Baker
Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam
Centre for Tropical Medicine and Global Health, Oxford University, Oxford, UK

*Corresponding author. Address: Oxford University Clinical Research Unit, 764 Vo Van Kiet, Ward 1, District 5, Ho Chi Minh City, Vietnam.
E-mail address: jcarrique-mas@oucru.org (J. Carrique-Mas)

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Article

Veterinary Drug Shops as Main Sources of Supply and Advice on Antimicrobials for Animal Use in the Mekong Delta of Vietnam

Doan Hoang Phu [1,2,*], Vu Thi Quynh Giao [1], Dinh Bao Truong [1,2], Nguyen Van Cuong [1], Bach Tuan Kiet [3], Vo Be Hien [3], Guy Thwaites [1,4], Jonathan Rushton [5] and Juan Carrique-Mas [1,4]

1 Oxford University Clinical Research Unit, HCMC, Vietnam; giaovtq@oucru.org (V.T.Q.G.); baotd@oucru.org (D.B.T.); cuongnv@oucru.org (N.V.C.); gthwaites@oucru.org (G.T.); jCarrique-Mas@oucru.org (J.C.-M.)
2 Faculty of Animal Science and Veterinary Medicine, Nong Lam University, HCMC, Vietnam
3 Sub Department of Animal Health, Cao Lanh city, Dong Thap, Vietnam; bachkiettydt1@gmail.com (B.T.K.); hienthuydt@gmail.com (V.B.H.)
4 Centre for Tropical Medicine and Global Health, Oxford University, Oxford OX3 7FZ, UK
5 Institute of Infection and Global Health, University of Liverpool, Liverpool L69 7BE, UK; J.Rushton@liverpool.ac.uk
* Correspondence: phudh@oucru.org; Tel.: +84-977-068-687

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Abstract: In the Mekong Delta of Vietnam, small-scale poultry farmers use large amounts of antimicrobials to raise their flocks, and veterinary drug shops owners and their staff are a key source of advice to farmers on antimicrobial use (AMU). We described the network of veterinary drug shops (n = 93) in two districts within Dong Thap province (Mekong Delta). We also interviewed a randomly selected sample of chicken farmers (n = 96) and described their linkages with veterinary drug shops. Antimicrobials represented 15.0% [inter quartile range (IQR) 6.0–25.0] of the shops' income. Fifty-seven percent shop owners had been/were affiliated to the veterinary authority, 57% provided diagnostic services. The median number of drug shops supplying antimicrobials to each farm during one production cycle was 2 [IQR 1–2]. Visited shops were located within a median distance of 3.96 km [IQR 1.98–5.85] to farms. Drug shops owned by persons affiliated to the veterinary authority that did not provide diagnostic services had a higher fraction of their income consisting of antimicrobial sales (β = 1.913; $p < 0.001$). These results suggest that interventions targeting veterinary drug shop owners and their staff aiming at improving their knowledge base on livestock/poultry diseases and their diagnosis may contribute to reducing overall levels of AMU in the area.

Keywords: veterinary drug shop; farmers; antimicrobial sales; Vietnam

1. Introduction

The indiscriminate use of antimicrobials in animal production is now recognised as a major driver of antimicrobial resistance (AMR) worldwide [1,2]. Approximately 80% of total antimicrobial usage (AMU) is thought to be aimed at animal production [3]. In the European Union, where data on AMU in humans and animals are regularly collated, AMU in animals represented 70.2% of a total of 12,720 tonnes used in 2014 [4]. Antimicrobials are used in animal production to treat and prevent disease, as well as for growth promotion in many countries [5,6]. In the Mekong Delta of Vietnam, veterinary drug shops are the main source of antimicrobials to chicken farmers [7].

A recent study showed that the density of veterinary drug shops at commune level was positively correlated with AMU in chicken flocks in this area [8]. In Vietnam, in 2015 there were ~10,400 licenced

veterinary drug shops (average 150–200 per province). There are approximately ~5000 licensed veterinary products on the market containing more than 70 antimicrobial ingredients [9]. The most significant recent development in the country is the launch (2017) of the National Action Plan (NAP) for the management of AMU and control of AMR in livestock production and aquaculture 2017–2020 [10]. The Vietnamese NAP is aligned with the Food and Agriculture Organisation Action Plan on AMR 2016–2020 [11] and includes key activities to support awareness, surveillance, governance and good AMU practices. However, the Vietnamese NAP does not specifically focus on the network of veterinary drug shops. As in many other low- and middle-income countries (LMICs) antimicrobials are sold 'over the counter' without a prescription [12].

High levels of AMU have been reported both in chicken and pig production in the Mekong Delta of Vietnam [13–15]. This behaviour is partly driven by the prevailing farming conditions that lead to a high incidence of disease and mortality [8]. In addition, farmers often use antimicrobials prophylactically as a replacement for good farming practices [16]. In the Mekong Delta of Vietnam, there are three to six veterinary drug shops per commune (~32 km^2), compared with only one or two government veterinarian/s or commune animal health worker/s. Farmers have more regular access to local veterinary drug shops than contact with any other animal health advisors [16]. It has been suggested that pharmacy owners should play a central role in antimicrobial stewardship [17,18]. Therefore, owners of veterinary drug shops and their staff are likely to play an important role in advising farmers on issues related to animal health, including AMU. However, it is of concern that owners and staff of these shops may have vested interests in the sale of antimicrobials. Here, the aims were: (1) To characterise and map out the veterinary drug shop network in the study area; (2) and to investigate linkages between veterinary drug shops with 96 randomly selected chicken farmers in a selected area of the Mekong Delta of Vietnam

2. Results

2.1. Characteristics of Veterinary Drug Shops

Of the 138 registered drug shops, 45 (32.6%) exclusively sold products for aquaculture. The owners of the remaining 93 veterinary drug shops (i.e., those targeting terrestrial animals) (50 in Cao Lanh, 43 in Thap Muoi) were interviewed. Demographic information and business activities of these shops are described in Table 1. Most (66.7%) owners were male and of a median age of 40 [IQR 36–51] years-old. The majority (59.1%) of veterinary shop owners had a vocational (animal science) qualification obtained in a technical college, whilst the remainder had a degree in veterinary medicine. One owner had a post-graduate (Master's) degree. Farmers were the main customer of these establishments (median 100% [IQR 90–100]), followed by animal health workers (mentioned by 36.6% veterinary drug shop owners). The median number of staff members (including the owner) working in each shop was 2 [IQR 1–2], the respective values were 2 [IQR 2–2] and 2 [IQR 1–2] for Cao Lanh and Thap Muoi. In term of staffing capacity, the median value was 12 person-days [IQR 7–14] per week, 14 [IQR 9–14] for Cao Lanh and 10 [7–14] for Thap Muoi drug shops. There were 5 (5.4%) shops staffed by personnel adding a total of 22.5–28.0 days/week. A total of 53 (57%) shop owners had links with the local veterinary authority (Sub-Department of Animal Health of Dong Thap, SDAH-DT) (50% in Cao Lanh and 65% in Thap Muoi). Of those, 6 (6.5%) shop owners were currently working at SDAH-DT. A total of 68.8% of shops provided loan services to farmers for specific goods, consisting of commercial feed (60.2% shops) and veterinary medicines (34.4%). Of the total income of antimicrobial sales, products for pig farming represented 30% [IQR 15–60%] income, followed by ducks (20% [IQR 13–40%]), chickens (15% [IQR 10–25]), aquatic species (0% [IQR 0–3]) and other (cows, goats) (2 [IQR 0–10]) respectively. Diagnostic services (including post-mortem necropsy) were available in 57% drug shops (76.0% in Cao Lanh and 34.8% in Thap Muoi).

Table 1. Descriptive characteristics of veterinary drug shops. The breakdown of total sample size into a fraction of antimicrobial sales is based on a median of 15% of antimicrobial sales.

Characteristics	All (n = 93)	<15% Antimicrobial Sales (n = 45)	≥15% Antimicrobial Sales (n = 48)
Owner's gender (%)			
Male	62 (66.7%)	31	31
Female	31 (33.3%)	14	17
Owner's age in year (median [IQR])	40 [36–51]	39 [36–48]	44 [38–52]
Education status (%)			
Master's degree	1 (1.1)	1	0
Degree in Vet Medicine/Animal husbandry	37 (39.8)	17	20
Vocational	55 (59.1)	27	28
District (%)			
Cao Lanh	50 (53.8)	29	21
Thap Muoi	43 (46.2)	16	27
Number of years in business (median [IQR])	13 [6–18]	12 [6–16]	14 [7–12]
Percent of customer by type (median [IQR])			
Farmer	100 [90–100]	100 [97–100]	100 [88–100]
Other vet shops	0 [0–0]	0 [0–0]	0 [0–0]
Animal health worker	0 [0–10]	0 [0–2]	0 [0–2]
Staffing (median [IQR])			
No. staff	2 [1–2]	2 [2–2]	2 [1–2]
Person-days per week	12 [7–14]	14 [9–14]	11 [7–14]
Affiliation (%)			
Veterinary authority (current)	6 (6.5%)	2	4
Veterinary authority (previous)	47 (50.5%)	19	28
No veterinary authority affiliation	40 (43.0%)	24	16
Diagnostic service available (%)			
Yes	53 (57.0%)	32	21
No	40 (43.0%)	13	27
Loan service (%)			
Any product (feed or health products)	64 (68.8)	32	32
Feed	56 (60.2)	31	25
Health products	32 (34.4)	14	18
Percent of antimicrobial sales by species (median/[IQR])			
Pig	30 [15–60]	30 [15–70]	30 [18–60]
Duck	20 [13–40]	25 [10–50]	20 [15–36]
Chicken	15 [10–25]	10 [10–20]	15 [10–25]
Aquatic animals	0 [0–3]	0 [0–3]	0 [0–5]
Other	2 [0–10]	4 [0–10]	1 [0–10]

The types of commodities and services offered by veterinary drug shop are displayed in Figure 1. A total of 85% shops retailed commercial feed, representing a median of 50.0% [IQR 20.0–70.0] of total income across shops. All shops dispensed antimicrobial products and other health-related products. Antimicrobial sales represented a median of 15.0% [IQR 6.0–25.0] of income. Non-antimicrobial drugs health-related products represented a median income of 16.0% [IQR 8.0–27.0] of income). A total of 11.8% and 3.2% veterinary drug shops respectively offered services, such as selling day-old chicks and collecting slaughtered-age chickens.

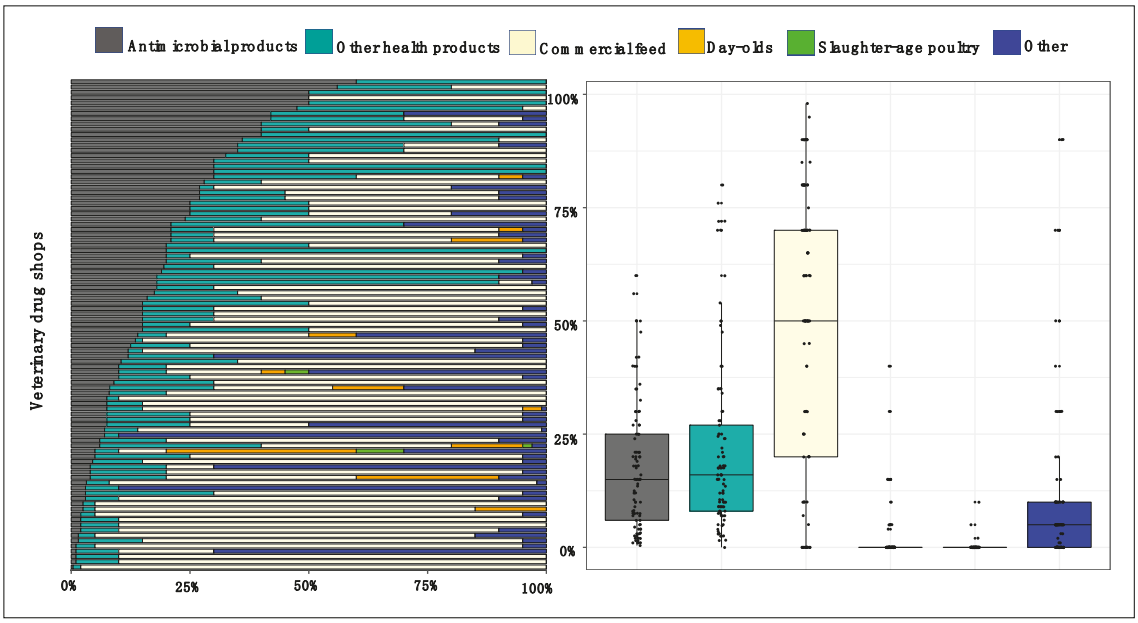

Figure 1. Types of commodities and services provided by veterinary drug shops in the study area. Each colour represents a type of commodity/service, including antimicrobials, non-antimicrobial health-supporting products, commercial feed, day-old-chicks, collection slaughter-age chickens, and 'Other' (services and other equipment: Drinkers, feeders, rice husks, etc.).

2.2. Mapping of Veterinary Drug Shops and Livestock Populations

The overall mean density of drug shops in the study area was 1.76 (SD ±1.94) per 10 km^2 (1.84/10 km^2 in Cao Lanh; 1.48/10 km^2 in Thap Muoi). Drug shops were unevenly distributed across the geographical space, with 5 clusters with a density of >5 shops/10 km^2 accounting for 47% of all shops (Figure 2). Overall, there were 745 ducks, 167 chickens, 36 pigs, and <8 of each other species (Muscovy ducks, goats, geese, cows etc.) per km^2 in the two study districts, representing an average of 2.5 tonnes of animal bodyweight per km^2 (2.4–2.6 in each study district).

Overall there were 3.8 shops (total 46.3 person-days) per 100 tonnes of animal bodyweight, 4.5 in Cao Lanh (57.2 person days), 3.2 (37.3 person-days) in Thap Muoi. These values were equivalent to 1 veterinary drug shop per 26.3 tonnes (~32,875 chickens/ducks or 669 pigs), and 1 person-day/week per 2.2 tonnes (~2750 chickens/ducks or 56 pigs) (Table 2).

2.3. Correlation between Number of Veterinary Drug Shops and Livestock Population

There were no significant correlations between the number of veterinary drug shops and the total animal bodyweight, as well as each of the three major species (duck, chicken and pig) at commune level. The Spearman's rank correlation coefficients were of 0.20, 0.19, 0.08 and 0.17 respectively, (all $p >$ 0.280). Similarly, the animal population of total bodyweight and three species of duck, chicken and pig were also not significantly correlated with the staffing capacity per commune (all $p > 0.520$), the correlation coefficients were 0.07, 0.12, 0.05 and 0.06 respectively. (Table S2, Figure S1).

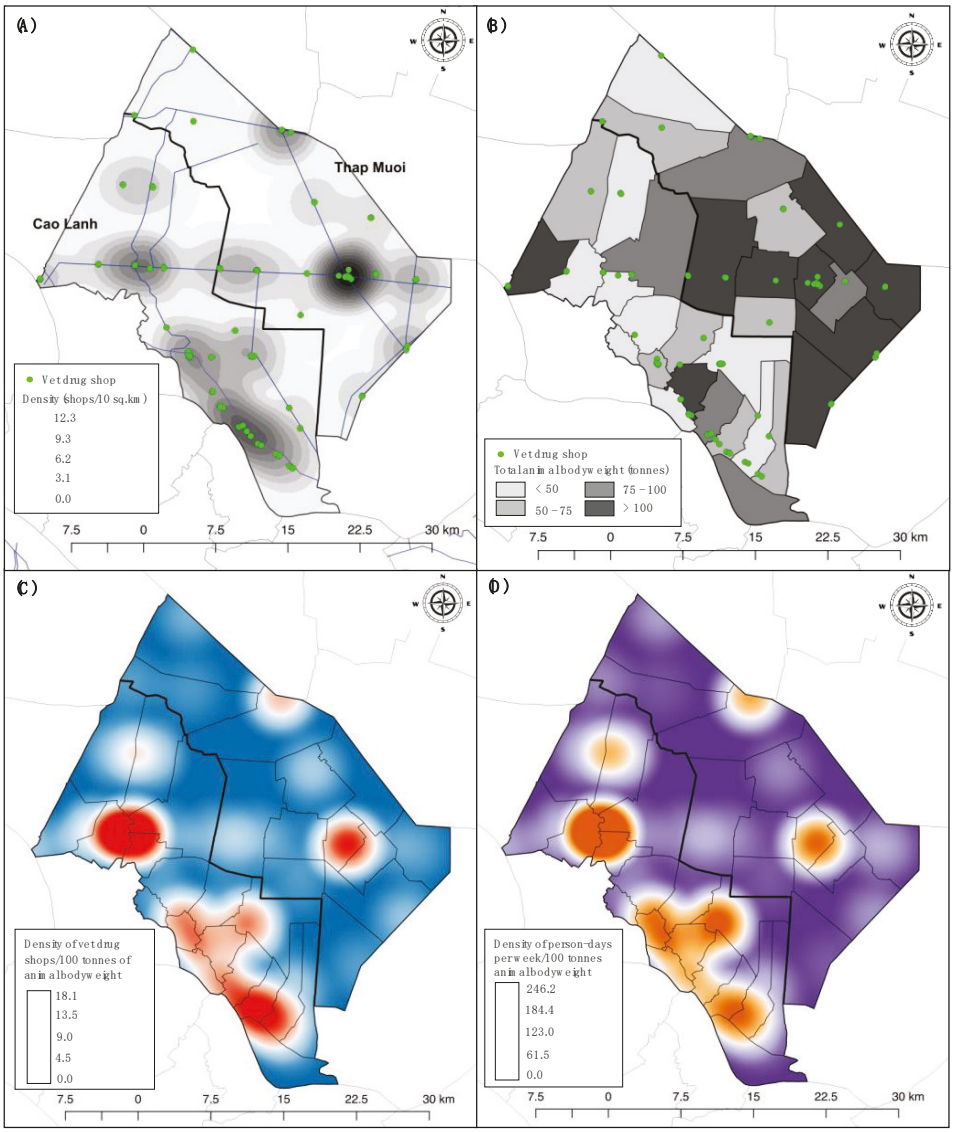

Figure 2. Geographical distribution of veterinary drug shop and livestock density at commune level in the study area. (**A**) Geographical distribution and kernel density of veterinary drug shops within an area of 10 km^2; (**B**) density of animal populations by commune expressed as animal bodyweight, and location of veterinary drug shops; (**C**) density heat map displaying the number of veterinary drug shops per 100 tonnes of animal bodyweight; (**D**) density heat map of staffing capacity (person-days/week) per 100 tonnes of animal bodyweight.

2.4. Linkages between Veterinary Drug Shops and Chicken Farms

A total of 96 chicken farmers were interviewed. The median number of veterinary drug shops visited by each farmer to purchase antimicrobials was 2 [IQR 1–2]. Farmers in Cao Lanh visited more veterinary drug shops than those in Thap Muoi (median 2 [IQR 2–2] vs. 1 [IQR 1–2] respectively) (Fisher's Exact Test, $p < 0.000$). Three farmers (3.1%) (2 in Cao Lanh, 1 in Thap Muoi) had visited >3 shops to buy antimicrobials, and 1 bought antimicrobial from 8 different veterinary drug shops. Two farmers had travelled outside their district for purchasing antimicrobials (Table S3). The distance between farms and the shops where farmers had purchased antimicrobials ranged from 0.03 to 14.97 km (median 3.96, [IQR 1.98–5.85]; 2.33 [IQR 1.43–4.36] for Cao Lanh, 5.15 [IQR 3.80–8.25] for Thap Muoi). Farms were located at a median distance of 1.92 km [IQR 0.96–2.76] from their closest veterinary drug shop (1.25 [IQR 0.73–2.06] for Cao Lanh, 2.53 [IQR 1.51–3.08] for Thap Muoi) (Figure 3).

Table 2. Veterinary drug shops and density of animal population by commune and district.

Commune	Area (km^2)	Vet Drug Shops	Person-Days per Week (Sum)	Person-Days per Week (Average)	Total Tonnes Animal Bodyweight	Tonnes Bodymass per km^2	No. of Drug Shops per 100 tonnes	Person-Days per Week per 100 tonnes
Cao Lanh District	464.7	50	635.0	12.7	1109.7	2.4	4.5	57.2
An Binh	8.2	0	0.0	0.0	28.9	3.5	0.0	0.0
Ba Sao	62.7	2	22.5	11.3	77.9	1.2	2.6	28.9
Binh Hanh Tay	14.4	6	63.0	10.5	60.7	4.2	9.9	103.9
Binh Hang Trung	19.9	4	31.5	7.9	95.6	4.8	4.2	32.9
Binh Thanh	27.6	0	0.0	0.0	75.5	2.7	0.0	0.0
Gao Giong	51.4	3	43.0	14.3	59.8	1.2	5.0	72.0
My Hiep	22.5	2	21.0	10.5	72.2	3.2	2.8	29.1
My Hoi	15.9	4	46.0	11.5	105.4	6.6	3.8	43.7
My Long	21.0	3	42.0	14.0	42.7	2.0	7.0	98.5
My Tho	23.9	1	7.0	7.0	66.8	2.8	1.5	10.5
My Tho town	8.2	5	75.0	15.0	59.2	7.2	8.4	126.7
My Xuong	9.9	2	30.5	15.3	39.5	4.0	5.1	77.1
Nhi My	26.0	1	14.0	14.0	49.2	1.9	2.0	28.4
Phong My	28.2	3	35.0	11.7	103.2	3.7	2.9	33.9
Phuong Thinh	44.3	2	38.0	19.0	45.1	1.0	4.4	84.2
Phuong Tra	17.5	6	81.5	13.4	33.1	1.9	18.1	246.2
Tan Hoi Trung	40.7	4	70.0	17.5	46.2	1.1	8.7	151.6
Tan Nghia	22.4	2	15.0	7.5	48.7	2.2	4.1	30.8
Thap Muoi District	517.7	43	502.0	11.7	1346.9	2.6	3.2	37.3
Doc Binh Kieu	32.6	4	73.5	18.8	216.0	6.6	1.9	34.0
Hung Thanh	49.6	1	7.0	7.0	74.0	1.5	1.4	9.5
Lang Bien	23.3	1	7.0	7.0	73.4	3.1	1.4	9.5
My An	18.6	1	9.5	9.5	97.4	5.2	1.0	9.8
My An town	17.1	12	149.5	12.5	107.9	6.3	11.1	138.6
My Dong	25.2	1	7.0	7.0	108.7	4.3	0.9	6.4
My Hoa	36.7	2	15.0	7.5	63.4	1.7	3.2	23.7
My Quy	61.3	6	63.0	10.5	112.0	1.8	5.4	56.2
Phu Dien	45.4	4	40.5	10.1	143.8	3.2	2.8	28.2
Tan Kieu	42.4	2	16.0	8.0	100.1	2.4	2.0	16.0
Thanh Loi	47.8	1	14.0	14.0	45.9	1.0	2.2	30.5
Thanh My	44.7	2	14.0	7.0	127.2	2.8	1.6	11.0
Truong Xuan	73.0	6	86.0	14.3	77.1	1.1	7.8	111.5
Whole study area	982.4	93	1137	12.2	2456.6	2.5	3.8	46.3

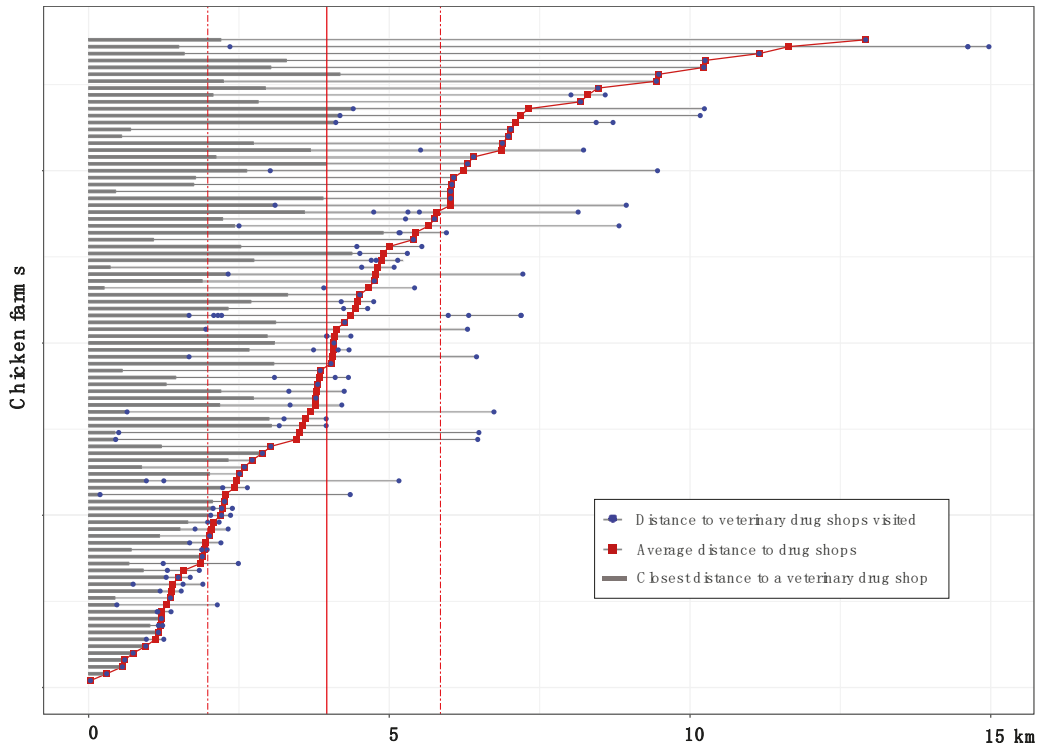

Figure 3. The geographical distance between farms (n = 94), their closest veterinary drug shop, and the visited veterinary drug shops from which farmers purchased antimicrobials. Data from 2 farms were excluded, since farmers visited other districts to buy antimicrobials. Solid red line: The median distance between farms and visited shops (3.96 km); Dashed red lines: Inter quartile range [1.98–5.85].

The reasons given by farmers to justify their choice of specific veterinary drug shops were, in decreasing order: (1) Animal health services (including advice on husbandry, diagnostic support, including post-mortem, vaccination support, sales of day-old-chicks) (mentioned by 47.9% farmers) (standardised score 38.5%); (2) Kinship (a relative or friend), mentioned by 37.5% farmers (score 31.7%); (3) Quality of products retailed (including the perception of being effective) (mentioned by 37.5% farmers (score 27.6%); (4) Distance from farm (26% farmers) (score 14.7%); and (5) price (7.2% farmers) (score 4.8%). Other, less commonly mentioned reasons (mentioned by 14.6% farmers in total) were: Knowledge, experience of qualification of the owner, availability of special products (drugs used in the brooding period) (scores 4.1) (Figure 4).

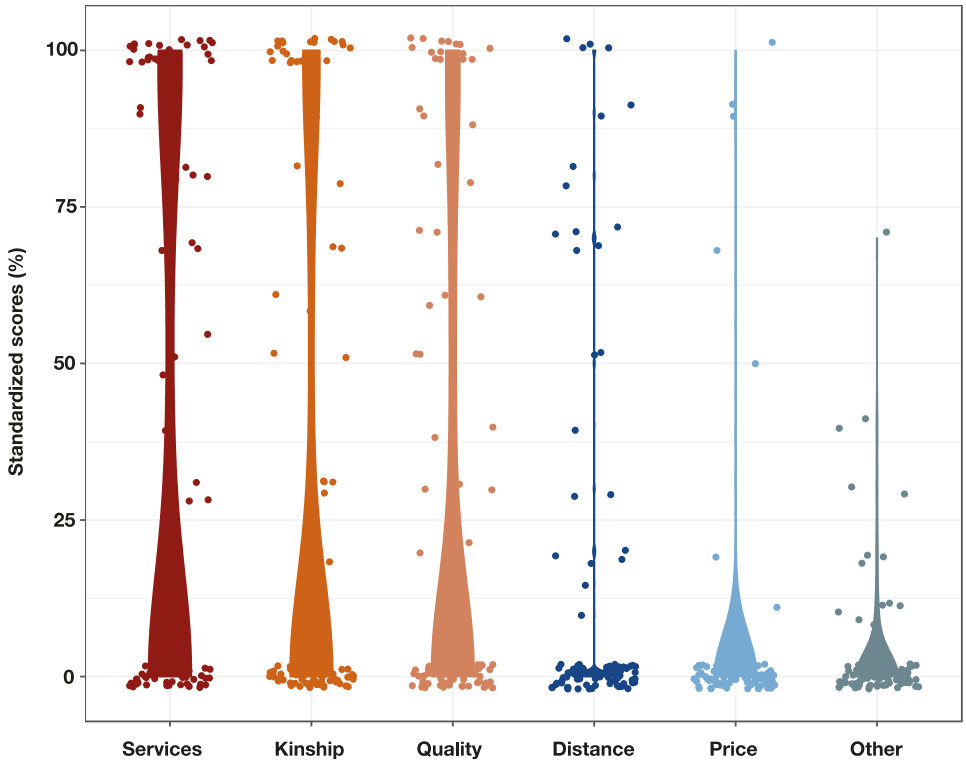

Figure 4. Standardised scores for the reasons stated by farmers for their choice of veterinary drug shops.

2.5. Risk Factor Analyses

Factors associated with a higher proportion of their income associated with antimicrobial sales in univariable models ($p < 0.2$) were: (1) Owner ≥ 40 years-old; (2) District of Thap Muoi; (3) Person-days per week (protective) (log), (4) Affiliation to government veterinary authority; and (5) Diagnostic services available. The latter two variables (Affiliation to government veterinary authority and Diagnostic services available) were combined into a new variable with four levels. In the final multivariable model, the variable 'District', 'Age', and 'Person-days' became non-significant, since they were confounded by affiliation to the veterinary authority. The highest risk corresponded to shops owned by veterinarians affiliated to the veterinary authority that did not provide diagnostic services (Table 3).

Table 3. Linear models that investigate factors associated with a higher share of income consisting of antimicrobial sales.

	Univariable			Multivariable *		
	β	95% CI	*p*-Value	*β*	95% CI	*p*-Value
Owner's gender (baseline = Male)						
Female	0.011	−0.77–0.79	0.976			
Owner's age (baseline <40 years old)						
≥40 years old	0.647	−0.07–1.37	0.079	0.156	−0.57–0.88	0.672
Education status (baseline = Vocational)						
Bachelor or higher	0.341	−0.40–1.08	0.366			
District (baseline = Cao Lanh)						
Thap Muoi	0.993	0.28–1.70	0.006	0.462	−0.31–1.24	0.241
No. of years in business (log)	0.207	−0.18–0.59	0.296			
Person-days per week (log)	−0.910	−1.81–0.00	0.049	−0.726	−1.62–0.16	0.110
Affiliation to veterinary government authority (baseline = No)						
Yes	0.773	0.04–1.50	0.037			
Diagnostic service (baseline = Yes)						
No	0.989	0.27–1.70	0.007			
Affiliation of shop owner and diagnostic service (baseline = No affiliation to veterinary government authority, diagnostic service)						
Affiliation to veterinary government authority, diagnostic service	0.656	−0.28–1.59	0.167	0.565	−0.38–1.51	0.239
Affiliation to veterinary government authority, no diagnostic service	1.913	0.88–2.94	<0.001	1.497	0.32–2.66	0.012
No affiliation to veterinary government authority, no diagnostic service	0.801	−0.25–1.85	0.135	0.692	−0.44–1.82	0.229
Loan service of any product (baseline = Yes)						
No	0.454	−0.33–1.24	0.257			
Kernel density of shops (log)	−0.087	−0.67–0.50	0.769			

* Intercept = 4.578; SE = 1.156.

3. Discussion

To our knowledge, this is the first study describing veterinary drug shops and their linkages with farmers in a low- and middle-income country. Our results indicate a poor spatial correlation between veterinary drug shops and animal populations at commune level. This is consistent with our observation that farmers often purchase antimicrobials from shops for reasons other than geographical proximity, preferring to travel to longer distances. In contrast, the provision of services (diagnostics, vaccination support, advice on flock health) were major factors influencing the farmers' choice/s of veterinary drug shop.

According to the farmers' opinions, antimicrobial retail prices had little impact on their specific choice of veterinary drug shop. This is consistent with a previous study conducted in the area, where poultry farmers stated that they would be willing to accept a three to four-fold hike in prices without altering their AMU behaviour [16]. It has been shown that antimicrobials intended for veterinary use are extremely affordable in the region (average of 0.56 cents of a USD per kilogram treated) and represent only a small fraction of overall chicken production costs [12]. We found that, despite high levels of AMU by chicken flocks in the area [15], antimicrobial sales represented a relatively small fraction of the total income of veterinary drug shops. There were, however, the large difference across establishments.

We found interesting differences in antimicrobial sales depending on the geographical location and the profile of the shop owner. Shops in Thap Muoi district obtained a higher fraction of their income from antimicrobial sales. However, this was explained by a higher fraction of drug shops in this district that did not offer diagnostic services. Interestingly, the provision of diagnostic services was not linked to the shop being owned by a fully qualified veterinarian (data not shown). Antimicrobials were more likely sold in establishments where diagnostic support services, even basic (i.e., post-mortem), were not available. This is consistent with studies in human medicine showing that uncertainty

of diagnosis or the absence of diagnostic facilities is factors leading to an excessive prescription of antimicrobials [19,20].

The higher density of pharmacies in Cao Lanh district is likely to result in competition among shop owners and be reflected in more likely availability of diagnostic services in their shops. Despite differences observed in antimicrobial sales and staff capacity in shops in these two districts, a previous study identified larger overall levels of AMU among chicken farmers in this district [8]. This was probably explained by a larger number of veterinary drug shops accessed by farmers in this district.

We mapped out veterinary drug shops and related these to animal bodymass. A previous study conducted in one district within Ho Chi Minh City estimated that there were 301 drug shops for a resident human population of 396,175 people [21]. Assuming an average bodyweight of 50 kilograms per person, we calculate that one pharmacy supplied to a total of 65.8 tonnes of human bodyweight. In contrast, in our study, there was one veterinary drug shop for 26.3 tonnes of animal bodyweight (i.e., 2.5 times higher than human drug shops).

Our study had a number of limitations: We only interviewed drug shop owners, even though other persons for which we did not gather information often staffed these shops. Also, there were a number of veterinary drug shops falling outside the district boundaries. This was more likely for farms located close to the edges of the district (data not shown). This may have resulted in an underestimation of the distances between farms and their chosen drug shops. We focused on small- scale chicken farms since small-scale farming is the most common type of farming system in the Mekong Delta and elsewhere in Southeast Asia. The chosen farms had already been enrolled as part of a large field-based project (www.viparc.org), and previous data indicated exceptionally high levels of AMU in these systems. We believe that, to a certain extent, our findings can be extrapolated to small farms raising other poultry species and pigs in the region.

4. Materials and Methods

4.1. Study Area, Populations and Veterinary Drug Shops

The study was conducted in two districts (Cao Lanh and Thap Muoi) within Dong Thap province (Mekong Delta of Vietnam) in October 2018. These two districts had a combined area of 982.6 km^2, representing 27% of the whole province, and have a combined population of 313,445 people (population density 319 people/km^2). Rice and fruit crops, as well as raising livestock (pigs, cattle, goats) and poultry (ducks, chickens and Muscovy ducks) are the main economic activities in this rural area. Data on animal populations by commune (an administrative sub-division within the district) were provided by the Sub-Department of Animal Health of Dong Thap (SDAH-DT) (official census, 2017). In Cao Lanh and Thap Muoi districts, there were a combined population of 732,337 ducks, 163,572 chickens, 35,647 pigs, 7843 Muscovy ducks, 2934 cows, 1160 goats, and 784 geese. A total of 138 active veterinary drug shops were registered in these two districts.

4.2. Correlation between Veterinary Drug Shop and Livestock Population

Data on the total number of animals (ducks, Muscovy ducks, chickens, pigs, geese, bovines, goats) in each commune were converted into animal bodyweight based on 50% of the average weight of slaughtered animals in Mekong region [22]: Duck, chicken (1.6 kg), goat (44.4 kg), pig (78.6 kg), cow (200 kg). The Muscovy duck and goose slaughter weights were estimated in 3.2 kg. We calculated the Spearman's rank correlation coefficient between the number of veterinary drug shops and animal bodyweight at commune level. Detailed on animal population by species at commune level are provided in Table S2.

4.3. Mapping of Veterinary Drug Shops and Livestock Density

The location coordinates of all veterinary drug shops and chicken farms in the two study districts were obtained. These were plotted using Quantum GIS (QGIS), version 2.18.15 (QGIS Development

Team) based on DIVA-GIS boundary data (https://www.diva-gis.org). A kernel density algorithm was used to create density heat maps of veterinary drug shop within a radius of 5 kilometers [23]. Likewise, the ratio of veterinary drug shops and person-days per week (the sum of working days of all staff including the shop owner per week) to total tonnes of animal bodyweight per commune was plotted using a kernel density.

4.4. Survey of Veterinary Drug Shops and Chicken Farmers

Veterinary drug shop owners in Cao Lanh and Thap Muoi district were interviewed using structured questionnaires. Information on demographic characteristics of the shop owners (i.e., age, gender, educational status) and other shop-related variables (district, number of years in business, type/s of customer, opening times, staffing capacity, types of products sold, diagnostic services, loan service of feed, health products and sales by species) were collected. In addition, we interviewed small-scale chicken farmers (raising between 100 and 2000 chickens) that had previously been randomly selected for a longitudinal study [24]. Farmers were asked to list the veterinary drug shops from where they purchased veterinary drugs over their latest flock production cycle. Farmers were asked to list and rank the reasons behind their choice of each drug shop, adding up to 100%. We calculated a standardised score for each reason by multiplying each of these ranks by the share of expenditure on each veterinary drug shop. The distances between veterinary drug shops and chicken farms were determined using the Distance Matrix Tool on QGIS.

4.5. Risk Factor Analysis

Risk factor analyses for the outcome variable 'proportion of business income consisting of antimicrobial sales (square root transformed) were carried out by linear regression. The variables investigated were: (1) Owner's gender; (2) Owner's age (as determined with a cut-off of median value of 40 years old); (3) Qualification of owner (vocational/bachelor or higher); (4) District; (5) Numbers of years in business (log); (6) Staffing capacity (person-days per week) (log); (7) Affiliation to veterinary authority (previous or current); (8) Diagnostic services available (including post-mortem); (9) Loan services available; (10) Kernel density of veterinary drug shop (log). A multivariable model was built using a step-wise forward approach to select the final model. Univariable models were screened, and those with a $p < 0.20$ were kept as a candidate for multivariable models. All statistical analyses were done using R (http://www.r-project.org).

5. Conclusions

Our findings suggest that improving the knowledge base of veterinary drug shops owners and their staff on animal diseases and diagnostics may contribute to reducing excessive dispensation of antimicrobials, whilst improving their awareness on the consequences of antimicrobial misuse. This should be coupled with more stringent licencing requirements and training certificates to owners of these shops, as well as any staff operating them.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-6382/8/4/195/s1, Figure S1: Correlation between animal population and number of veterinary drug shops/ total person-day per week at commune level, Table S1: Data on survey of veterinary drug shop, Table S2: Total animal population in study area, Table S3: Geographical coordinates and distance between veterinary drug shops and chicken farms, Table S4. Standardised scores of reasons for choosing a veterinary drug shop by chicken farmers.

Author Contributions: D.H.P., V.T.Q.G., J.C.-M. designed the study. D.H.P., V.T.Q.G. conducted field surveys. B.T.K., V.B.H. designed and aided data collection. D.H.P., J.C.-M., D.B.T., N.V.C. contributed to data analyses. D.H.P., J.C.-M., V.T.Q.G. contributed to draft manuscript. J.R., G.T., N.V.C., B.D.T. reviewed the results and drafted of manuscript. The manuscript has been read and approved by all authors.

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ORIGINAL ARTICLE

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Labelling and quality of antimicrobial products used in chicken flocks in the Mekong Delta of Vietnam

Nguyen Thi Phuong Yen[1] | Doan Hoang Phu[1] | Nguyen Van Cuong[1†] | Bach Tuan Kiet[2] | Be Vo Hien[2] | Pawin Padungtod[3] | Dinh Bao Truong[1,4] | Guy E. Thwaites[1,5] | Juan J. Carrique-Mas[1,5]

[1]Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

[2]Sub-Department of Animal Health and Production, Cao Lanh, Dong Thap Province, Vietnam

[3]Emergency Center for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations, Hanoi, Vietnam

[4]Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh, Vietnam

[5]Nuffield Department of Medicine, Oxford University, Headington, Oxford, United Kingdom

Correspondence
Juan J. Carrique-Mas, Oxford University Clinical Research Unit, 764 Vo Van Kiet, Ho Chi Minh City, Vietnam.
Email: jcarrique-mas@oucru.org

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Abstract

Background: The Mekong Delta of Vietnam is a hotspot of antimicrobial use (AMU), but there is no information on the quality of the labelling and strength of antimicrobial products used in poultry production.

Methods: Based on a large random sample of farms, we identified the 20 most used antimicrobial products in the area, and investigated their antimicrobial active ingredient (AAI) content by UPLC-MS/MS (91 analytical tests).

Results: Only 17/59 (28.8%) batches contained all AAIs within 10% of the declared strength. Worryingly, 65.0% products provided in their label preparation guidelines for both therapeutic and prophylactic use. Withdrawal times for both meat and eggs were stated in 8/20 (40%) products.

Conclusion: Results highlight deficiencies in quality and labelling contents that undermine authorities' efforts to discourage inappropriate use of antimicrobials.

KEYWORDS
animal production, antimicrobials, poultry, veterinary, Vietnam

1 | INTRODUCTION

Antimicrobials are widely used in animal production, both to prevent and to treat diseases. In some countries, antimicrobials are also added to commercial feed formulations to promote rapid growth (Page & Gautier, 2012). It has been estimated that in African countries about 50% of antimicrobials available in the market correspond to non-standard and non-registered veterinary medicines (Clifford et al., 2018). There is a concern that inadequate formulation of these products may lead to exposure to sub-therapeutic levels of antimicrobials, therefore promoting resistance among bacterial populations (Nwokike, Clark, & Nguyen, 2018). Recent studies on the quality of antimicrobial products used in shrimp and catfish farming in Vietnam indicated that only ~8% and ~29% products contained an AAI within ±10% (accepted level of variation) (Phu, Phuong, Scippo, & Dalsgaard, 2015; Tran, Tran, Phan, & Dalsgaard, 2018). Globally, the quantity of antimicrobials used in chicken production is estimated at 138.0 doses/1,000 animal-days [inter quartile range (IQR)

[†]Correction added on 11 September 2019, after first online publication: the first name and surname for the third author were interchanged in the original publication; this has now been corrected to Nguyen Van Cuong.

TABLE 1 Characteristics of 20 antimicrobial products investigated, sorted by the number of flocks where they were used

Product code	No. flocks used (%)	Company	Package content	Target species	Declared strength (%=g/100 g product)	Indication	Product description	Withdrawal time indicated (meat, eggs)
AP01	34.5	A	100 g	Chickens, ducks	OTC 1.5%; COL 0.07%	Prophylactic, therapeutic	Antimicrobial mixed with vitamins	Meat
AP02	14.8	F	20 g	Poultry, ruminants, pigs, horses	AMX 10%; COL 2%	Therapeutic	Antimicrobial only	Meat, eggs
AP03	14.8	A	100 g	Poultry	OTC 10%; STR 5%	Therapeutic	Antimicrobial only	Meat
AP04	11.8	G	50 g	Poultry	OTC 4%; COL 2%	Prophylactic, therapeutic	Antimicrobial mixed with vitamins	No
AP05	11.8	G	100 g	Poultry, ruminants, pigs	TAP 6%; SMZ 5%	Prophylactic, therapeutic	Antimicrobial only	Meat
AP06	10.8	D	100 g	Poultry, ruminants, pigs	OTC 5%; COL 0.017%	Prophylactic, therapeutic	Antimicrobial mixed with vitamins	Meat, eggs
AP07	8.4	B	100 g	Poultry, pigs	OTC 7%; COL 0.98%	Prophylactic, therapeutic	Antimicrobial only	Meat, eggs
AP08	8.4	G	100 g	Poultry, ruminants, pigs	TYL 7%; GEN 3.5%	Prophylactic, therapeutic	Antimicrobial mixed with minerals	Meat, eggs
AP09	7.4	H	100 g	Poultry, ruminants, pigs	DOX 20%; TYL 10%	Prophylactic, therapeutic	Antimicrobial only	Meat, eggs
AP10	5.9	G	100 g	Poultry	ERY 6%; SMZ 10%	Prophylactic, therapeutic	Antimicrobial only	Meat
AP11	5.4	C	50 g	All animal species	GEN 6%; COL 2.44%	Therapeutic	Antimicrobial mixed with vitamins, anti-inflammatory	Meat, eggs
AP12	5.4	G	100 mL	Poultry, ruminants, pigs	TIL 25%	Prophylactic, therapeutic	Antimicrobial only	Meat
AP13	5.4	H	100 g	Poultry, ruminants, pigs	ENR 5%	Prophylactic, therapeutic	Antimicrobial mixed with expectorant, analgesic	Meat
AP14	4.9	I	100 g	Ducks, Muscovy ducks	ENR 5%	Prophylactic, therapeutic	Antimicrobial only	Meat
AP15	4.4	I	100 g	Poultry	AMX 10%; TYL 10%	Prophylactic, therapeutic	Antimicrobial mixed with expectorant, analgesic	Meat
AP16	3.9	A	100 g	Poultry, ruminants, pigs	OTC 1%	Not explicit	Antimicrobial mixed with vitamin, analgesic, antipyretic	Meat, eggs
AP17	3.9	I	100 g	Poultry, ruminants, pigs	NEO 6%; COL 1.46%	Therapeutic	Antimicrobial only	Meat

(Continues)

TABLE 1 (Continued)

Product code	No. flocks used (%)	Company	Package content	Target species	Declared strength (%=g/100 g product)	Indication	Product description	Withdrawal time indicated (meat, eggs)
AP18	3.4	I	100 g	Poultry, ruminants, pigs	TMP 3%; COL 2%	Therapeutic	Antimicrobial only	Meat, eggs
AP19	3.0	E	100 g	Poultry	DOX 2.5%; TYL 2.5%	Prophylactic, therapeutic	Antimicrobial mixed with vitamin	Meat
AP20	1.0	C	50 g	Poultry, ruminants, pigs	GEN 3%; TYL 5%	Therapeutic	Antimicrobial only	Meat

Abbreviations: AMX, amoxicillin; COL, colistin; DOX, doxycycline; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; NEO, neomycin; OTC, oxytetracycline; SMZ, sulphametoxazole; STR, streptomycin; TAP, thiamphenicol; TIL, tilmicosin; TYL, tylosin.

91.1–438.3], a higher amount than AMU in the two other major terrestrial food animal species (pig and cattle) (Cuong, Padungtod, Thwaites, & Carrique-Mas, 2019). Previous studies have reported exceptionally high levels of antimicrobial use (AMU) in chicken farms in the Mekong Delta region of Vietnam (Carrique-Mas et al., 2015; Carrique-Mas et al., 2019; Cuong et al., 2019; Nguyen et al., 2016). However, there are currently no published data on the quality of antimicrobial products used in these farming systems. We investigated the labelling and strength of AAIs of the most commonly used products in representative chicken farms in the Mekong Delta of Vietnam.

Antimicrobial products were identified from a survey of 102 randomly selected farms raising meat chickens in Dong Thap province from November 2016 to March 2018. A total of 203 flocks raised in those farms with a completed full cycle of production were included in the study (Carrique-Mas & Rushton, 2017; Cuong et al., 2019). All flocks consisted of native breed chickens raised over a median period of 18 [Interquartile Range 16-20] weeks, with birds typically raised using all-in-all-out system. At the beginning of the project, farmers were given purposefully designed diaries to record their AMU, as well as containers where farmers were asked to store all packages of antimicrobials. A team of trained animal health workers visited each farm four times during each production cycle to review the collected data. The 20 most frequently used antimicrobial products were identified. Three different batches of each product were purchased from veterinary drug shops within the province of Dong Thap. The 20 most commonly used antimicrobial-containing products (defined as the proportion of flocks using) were identified, and information on strength on AAIs, species target, prophylactic/therapeutic indication, and withdrawal times for meat and egg productions was compiled. The products' contents were tested (single blinded) for the presence and strength of the AAIs declared in the label at an accredited laboratory (Center for Analysis Service of Experiment, Ho Chi Minh City, ISO 9001:2008 accredited) using Ultra High Performance Liquid Chromatography coupled to tandem Mass Spectrometry (UPLC-MS/MS). Three aminoglycoside antimicrobials (gentamicin, neomycin and streptomycin) were not investigated. For colistin, the number of International Units (IU) indicated in the label was converted to miligrams. Results were expressed as a percent of the declared strength indicated in the label (percent content). The inter-batch variability (in relation to the overall variability) was investigated by fitting a null random effects model with product fitted as a random effect and percent content as the outcome using lme4 package and R software.

The 20 products identified were marketed by nine different companies, and all except one (a French company selling product AB008) were Vietnamese (Table 1). All products were formulated for oral administration: Nineteen (95%) were powder-based formulations and one (5%) was a liquid solution. Five (25%) products contained a single antimcrobial and 16 (75%) a mixture of two antimicrobials. In order to investigate the inter-batch variability, three batches of 19 products and two batches of one product (AB051) were investigated, making a total of 91 analytical tests (Table 1).

Twelve different AAIs were identified in the 20 products, the most common being: colistin (8 products), oxytetracycline (6), gentamicin (2), tylosin (2), doxycycline (2), amoxicillin (2) and enrofloxacin (2). Other AAIs (trimethoprim, streptomycin, tilmicosin, erythromycin and neomycin) were contained in one product each. Six of those AAIs (colistin, gentamicin, tylosin, erythromycin, tilmicosin and neomycin) are considered to be critically important antimicrobials according to the World Health Organization (Anon 2017).

In six (30.0%) products the label provided an explicit indication for therapeutic administration only, 13 (65.0%) products provided an indication for both therapeutic and prophylactic use, and one (5.0%) did not include any indication. Withdrawal times for both egg and meat production were provided in the labels of eight (40.0%) products; in 11 (55.0%) products withdrawal times were indicated only for meat (but not for eggs); one product contained no indications with respect to withdrawal time. A total of 11 (55.0%) products contained only one AAI, and the remaining had other substances (including vitamins, mineral supplements and expectorants and analgesic substances). Twenty-eight (30.8%) samples tested were within 10% of the strength declared in the label. Thirty-four (37.4%) contained AAIs above the declared upper limit, and 27 (29.7%) below the declared lower limit. Two extreme values were observed for two AAIs: one (Product AP16) contained oxytetracycline with strength ranging from 10.3% to 11.9% and another (AB09) product had doxycycline strength ranging from 141.5% to 165.0% of the stated value (Figure 1).

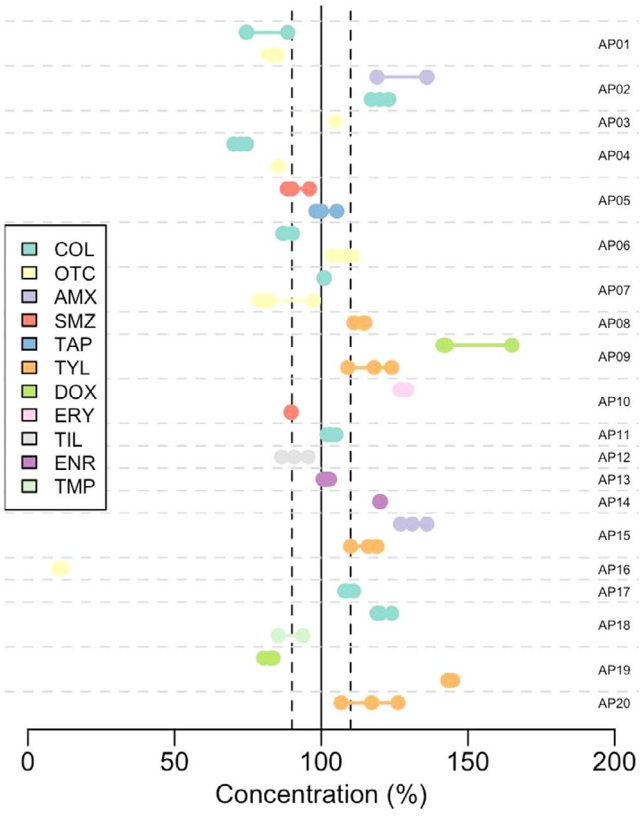

FIGURE 1 Results of the analyses of strength of antimicrobial AAIs in the 20 most commonly used products in poultry farms in the Mekong Delta of Vietnam. Products are sorted by decreasing prevalence of use by flock. Each dot across horizontal line corresponds to the results of the concentration of one AAI analysed

In 27/91 (29.7%) of the tests conducted the AAIs had a strength below the acceptable lower limit (−10%). Unexpectedly, 34/91 (37.4%) had AAIs with strength higher than that indicated in the label. Of the 59 individual product batches investigated, only 17 (28.8%) had all their AAIs within the ±10% acceptable range. Only 3 of the 20 (15.0%) products had all batches and all their AAIs within the ±10% range. A total of 24.5% of the variance was attributed to between-batch variation, the remainder being due to between-product variation.

Since our study is based on a random sample of farms, we are confident that these results are representative of antimicrobial products most commonly used by poultry farmers in the Mekong Delta of Vietnam. Currently there are >10,000 licensed veterinary products in the country, of which about ~50% consist of antibacterial antimicrobial formulations (Anon 2016). This makes quality control monitoring extremely challenging, particularly in a limited-resource setting such as Vietnam.

Quality testing of AAIs is very costly, and there is a lack of unbiased information about this issue in animal production in most countries. It has been previously estimated that one in 10 medicinal products in low- and middle-income countries is substandard or falsified (Nwokike et al., 2018). Given that the identity of antimicrobials declared in the label was confirmed in all cases, we do not believe that outright falsification is a major issue here. Furthermore, 'legal' antimicrobials are currently very affordable in Vietnam, and two-thirds of the products investigated had an indication for 'prophylactic use' in the label (normally followed by a list of bacterial diseases). This labelling openly conflicts with the animal health authorities' efforts to discourage routine use of antimicrobials for preventing disease (Aidara-Kane et al., 2018; Anon 2013) and sends a 'wrong' message to farmers (the end users), who will not be able unable to discern in the few instances that medication may be required in the absence of disease. This is particularly relevant in the context of small-scale farmers in many low- and middle-income countries. Farmers in these settings often do not have access to veterinary services capable of providing them with unbiased advice on AMU.

Under dosing is expected to result because of either sub-optimal quality of the manufactured product, or inadequate preparation at the point of administration by the farmer. For most products, the guidelines for product preparation (mixing with water) for prophylaxis were about half the strength required for therapeutic purposes. There is a risk that this may increase the probability of selection of AMR in bacterial populations (Ungemach, Mueller-Bahrdt, & Abraham, 2006). Withdrawal times for egg production were not specified in 60% of the antimicrobial products investigated. This is a concern, since these products are likely to be used both in meat and layer flocks. The observed inter-batch variation in product quality suggests deficiencies in the mixing/packaging process, since in Vietnam most AAIs sold in Vietnam are bulk-imported and then mixed, packaged and distributed within the country.

Based on a representative field survey, we identified the most common antimicrobial products used in poultry farming in the

Mekong Delta. Results indicate variable quality results, with only 17 (28.8%) product batches containing AAIs within the acceptable ±10% range. In addition to improving quality control of veterinary medicine products, we strongly advocate for enhancing regulation and inspection of antimicrobial product labelling, crucially removing the indication for prophylactic use. In all cases, products should indicate withdrawal times for meat, eggs and milk (for products aimed at ruminants). It would be desirable to limit the access to antimicrobials of critical importance for human health for veterinary use, and therefore development of policies aiming at this should be a priority.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required, as this is a retail study, with no direct implications or impact on any particular subject.

ORCID

Juan J. Carrique-Mas https://orcid.org/0000-0001-9161-8890

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Globalization and Health

RESEARCH Open Access

A survey of retail prices of antimicrobial products used in small-scale chicken farms in the Mekong Delta of Vietnam

Nguyen T. T. Dung[1,2], Bao D. Truong[1,3], Nguyen V. Cuong[1], Nguyen T. B. Van[1], Doan H. Phu[3], Bach T. Kiet[4], Chalalai Rueanghiran[5], Vo B. Hien[4], Guy Thwaites[1,7], Jonathan Rushton[6] and Juan Carrique-Mas[1,7*]

Abstract

Background: In the Mekong Delta region of Vietnam, high quantities of products containing antimicrobial are used as prophylactic and curative treatments in small-scale chicken flocks. A large number of these contain antimicrobial active ingredients (AAIs) considered of 'critical importance' for human medicine according to the World Health Organization (WHO). However, little is known about the retail prices of these products and variables associated with the expense on antimicrobials at farm level. Therefore, the aims of the study were: (1) to investigate the retail price of antimicrobials with regards to WHO importance criteria; and (2) to quantify the antimicrobial expense incurred in raising chicken flocks. We investigated 102 randomly-selected small-scale farms raising meat chickens (100–2000 per flock cycle) in two districts in Dong Thap (Mekong Delta) over 203 flock production cycles raised in these farms. Farmers were asked to record the retail prices and amounts of antimicrobial used.

Results: A total of 214 different antimicrobial-containing products were identified. These contained 37 different AAIs belonging to 13 classes. Over half (60.3%) products contained 1 highest priority, critically important AAI, and 38.8% 1 high priority, critically important AAI. The average (farm-adjusted) retail price of a daily dose administered to a 1 kg bird across products was 0.40 cents of 1 US$ (¢) (SE ± 0.05). The most expensive products were those that included at least one high priority, critically important AAI, as well as those purchased in one of the two study districts. Farmers spent on average of ¢3.91 (SE ± 0.01) on antimicrobials per bird over the production cycle. The expense on antimicrobials in weeks with disease and low mortality was greater than on weeks with disease and high mortality, suggesting that antimicrobial use had a beneficial impact on disease outcomes ($\chi^2 = 3.8$; $p = 0.052$). Farmers generally used more expensive antimicrobials on older flocks.

Conclusions and recommendation: The retail prices of antimicrobial products used in chicken production in Mekong Delta small-scale chicken farms are very low, and not related to their relevance for human medicine. Farmers, however, demonstrated a degree of sensitivity to prices of antimicrobial products. Therefore, revising pricing policies of antimicrobial products remains a potential option to curb the use of antimicrobials of critical importance in animal production.

Keywords: Mekong Delta, Vietnam, Chicken, Poultry, Antimicrobial, Animal daily dose, Cost

* Correspondence: jcarrique-mas@oucru.org
[1]Wellcome Vietnam Africa Asia Program, Oxford University Clinical Research Unit, 764, Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam
[7]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, Oxford University, Oxford, UK
Full list of author information is available at the end of the article

Background

Antimicrobial resistance (AMR) is a global health concern and excessive antimicrobial use (AMU) in animal production is one of the contributing factors [1]. The AMR situation has reached critical levels, and countries are being urged to take immediate action to mitigate the problem [2]. The practice of purchasing antimicrobials 'over the counter' without a prescription is widespread in many low- and middle-income countries (LMICs) [3]. This is also common practice in Vietnam, a country that currently ranks as the 15th most populous in the world (~ 97 M in 2019), in spite of existing legislation restricting access to antimicrobials for human use without prescription [4]. In contrast, antimicrobials intended for animal use can be legally purchased without a prescription by anyone from any of the approximately 10,000 veterinary drug shops across the country [5].

In 2011, the World Health Organization of the United Nations (WHO) ranked antimicrobial active ingredients (AAI) based on prioritization criteria for human medicine. This list has been modified on several occasions, and in 2018 the highest priority, critically important AAI category included 3rd and 4th generation cephalosporins, glycopeptides, macrolides, ketolides, polymyxins and quinolones [6].

There is growing consensus that use of antimicrobials of critical importance for human medicine in animals should be restricted/reduced [7–9]. However, a large number of AAIs considered by WHO to be of critical importance are currently used in animal production worldwide [10]. The Mekong Delta of Vietnam is regarded as a hotspot for AMU in animal production [11–14], and levels of use of AAIs considered of critical importance are high. A recent study on small-scale chicken farms in the Mekong Delta indicated that 76.2% antimicrobial products contained AAIs of critical importance according to WHO [14]. It has been suggested that antimicrobials used in animal production in Vietnam are very affordable. A study on the 10 most popular products used by farmers showed that the average cost of a daily dose was 0.56 cents of 1 US$ (range ranged from 0.19 to 1.03) [15]. It is not clear whether retail prices reflect their AAIs composition and their relevance to human health, and to what extent low pricing contributes to excessive AMU in animal production in Vietnam. We investigated antimicrobial products used in a sample of 112 randomly selected small-scale commercial farms (203 flocks) raising native chickens in the Mekong Delta of Vietnam. We quantified AAIs contained in these products as well as their retail prices. The aims of this study were: (1) to investigate the price of antimicrobials with regards to WHO importance criteria and formulation (single AAIs or combined AAIs) and (2) to investigate changes in the expense on antimicrobials over the production cycle.

Results

Total and weekly expense on antimicrobials

Data on AMU and their retail prices were obtained from 203 complete cycles of native chicken flocks raised for meat in 102 farms. The median flock size at restocking was 300 [Inter-quartile range (IQR) 200–495], and the median duration of production cycles was 18 [IQR 16–20] weeks. The median cumulative mortality over the whole production cycle across flocks was 14.10% [IQR 6.8–29.2]. The average probability of AMU by week across flock cycles was 0.21 (SE ± 0.02) (Fig. 1a). The total expense on antimicrobials by farmers over the 203 cycles of production was US$2529.50 (Fig. 1b). The average expense on antimicrobials per flock cycle was US$12.50. The average cumulative expense on antimicrobials to raise one bird was ¢3.91 (SE ± 0.03). On average, farmers spent ¢64.07 (SE ± 2.45) on antimicrobials per week (Fig. 1b), and ¢0.20 (SE ± 0.01) per bird per week (Fig. 1c).

The highest probability of AMU corresponded to the first week of age of flocks (0.76; SE ± 0.03), decreasing thereafter (Fig. 1a). The weekly total expense on antimicrobials was highest during the 8–12 period week, peaking on week 10 (per flock mean ¢128.60; SE ±13.36) (Fig. 1b). After week 13, overall expense on antimicrobials decreased considerably (≤¢52.19 per week). In relation to live chicken weight, the weekly average expense on antimicrobials was was ¢0.75 per kg of live bird (SE ± 0.05). The highest expense corresponded to the first week of production (per flock mean ¢6.36; SE ±0.35) and quickly decreasing thereafter (≤¢2.21 per week) (Fig. 1d).

Antimicrobials and disease

The probability of disease was highest during the first week of the production cycle (0.56 SE ± 0.02), decreasing thereafter. Overall bird mortality peaked during the 5–10 week period (Fig. 2a). Of a total of 3948 weeks observed across all of 203 flocks, 1113 (28.19%) corresponded to weeks with disease (clinical signs reported) and 2835 (71.81%) to weeks without disease.

On average, farmers spent ¢125.38 (SE ± 6.76) and ¢40.0 (SE ± 1.97) on antimicrobials on their flocks in weeks with and without disease, respectively (Kruskal-Wallis $\chi^2 = 367.3$; $p < 0.001$). The (average) expense (per bird) on antimicrobials in weeks with and without disease was ¢0.34 (SE ± 0.02) and ¢0.15 (SE ± 0.01), respectively (Kruskal-Wallis $\chi^2 = 315.7$; $p < 0.001$). Of weeks with disease, the highest overall expense on antimicrobials corresponded to weeks 8–12, with a peak in week 10 (flock mean ¢210.81; SE ± 39.75) (Fig. 2b). Weekly mortality was categorized as low or high based on a (mean) cut-off of 2.8 per 100 birds (2.8%). The average expense on antimicrobials (per flock) in weeks with disease with high and low mortality was ¢137.86 (SE ± 8.34)

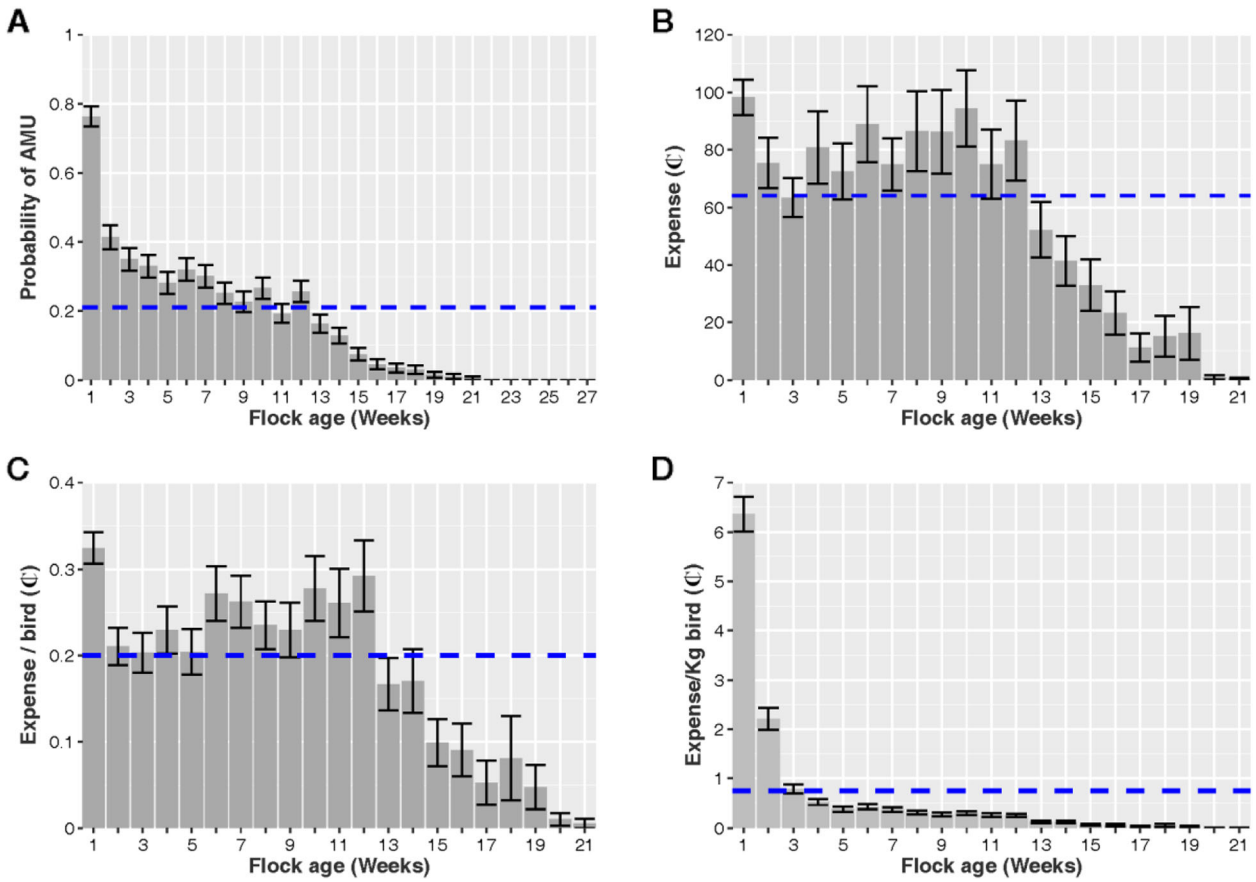

Fig. 1 **a** Probability of flocks using antimicrobials by age (weeks); **b** Weekly expense (per flock) on antimicrobials during the flock production cycle by age; **c** Total expense (per bird) on antimicrobials; **d** Total expense per kilogram of bird. Costs are expressed in cents of 1 US$ (¢). The blue lines represent the crude (unadjusted) mean across all observation weeks. Vertical bars represent the standard error of the mean (SE)

and ¢157.40 (SE ± 7.98), respectively (Kruskal-Wallis $\chi^2 = 2.9$; $p = 0.085$) (Fig. 2c). The equivalent per bird expense was ¢0.37 (SE ± 0.02) and ¢0.47 (SE ± 0.03) for weeks with, respectively, high and with low mortality (Kruskal-Wallis $\chi^2 = 1.2$; $p = 0.274$). The average cost of antimicrobial products used (expressed as cost of product ADD_{kg}) chosen in weeks with and without disease was, respectively, ¢0.60 (SE ± 0.04) and ¢0.54 (SE ±0.05) (Kruskal-Wallis $\chi^2 = 0.4$; $p = 0.528$) (Fig. 2d).

Retail prices of antimicrobial products and AAIs

Retail prices of antimicrobial-containing products were collated from farmers' records documenting AMU on 191 full flock cycles (raised in 100 farms). A total of 619 different health-supporting products were identified, of which 236 products contained AAIs. Data on 22 antimicrobial-containing products intended for human use (tablets) and injectable antimicrobials for animal use were excluded, since it was not clear how these were administered to flocks, the quantities used and number of birds treated. A total of 775 pricing records on the remaining 214 antimicrobial products were used to summarize retail prices. These 214 products contained 37 different AAIs belonging to 13 classes. A total of 71.9% products contained only antimicrobial active ingredients (AAIs) (apart from excipient), whilst 28.1% contained AAIs mixed with substances such as vitamins, mineral and electrolytes. Examination of the products' labels indicated that 76 contained one AAI, 137 contained two AAIs, and one contained four AAIs (tylosin, sulphamethoxazole, sulfadiazine, and trimethoprim) (used by one farm on one flock) An additional file gives a detailed description of all 214 products (Additional file 1). Data from a total of 775 price estimates from farmers were used to summarize the price of products based on their AAI composition (see Additional file 2). A table with information on all antimicrobial products broken down by their AAIs content, the number of farms using these products and their mean retail price is given in an additional file (see Additional file 3). These are further aggregated by class of AAI in Table 1.

A total of 129 (60.3%) products contained at least one critically important of the highest priority AAI; 82 (38.3%) contained at least one critically important of

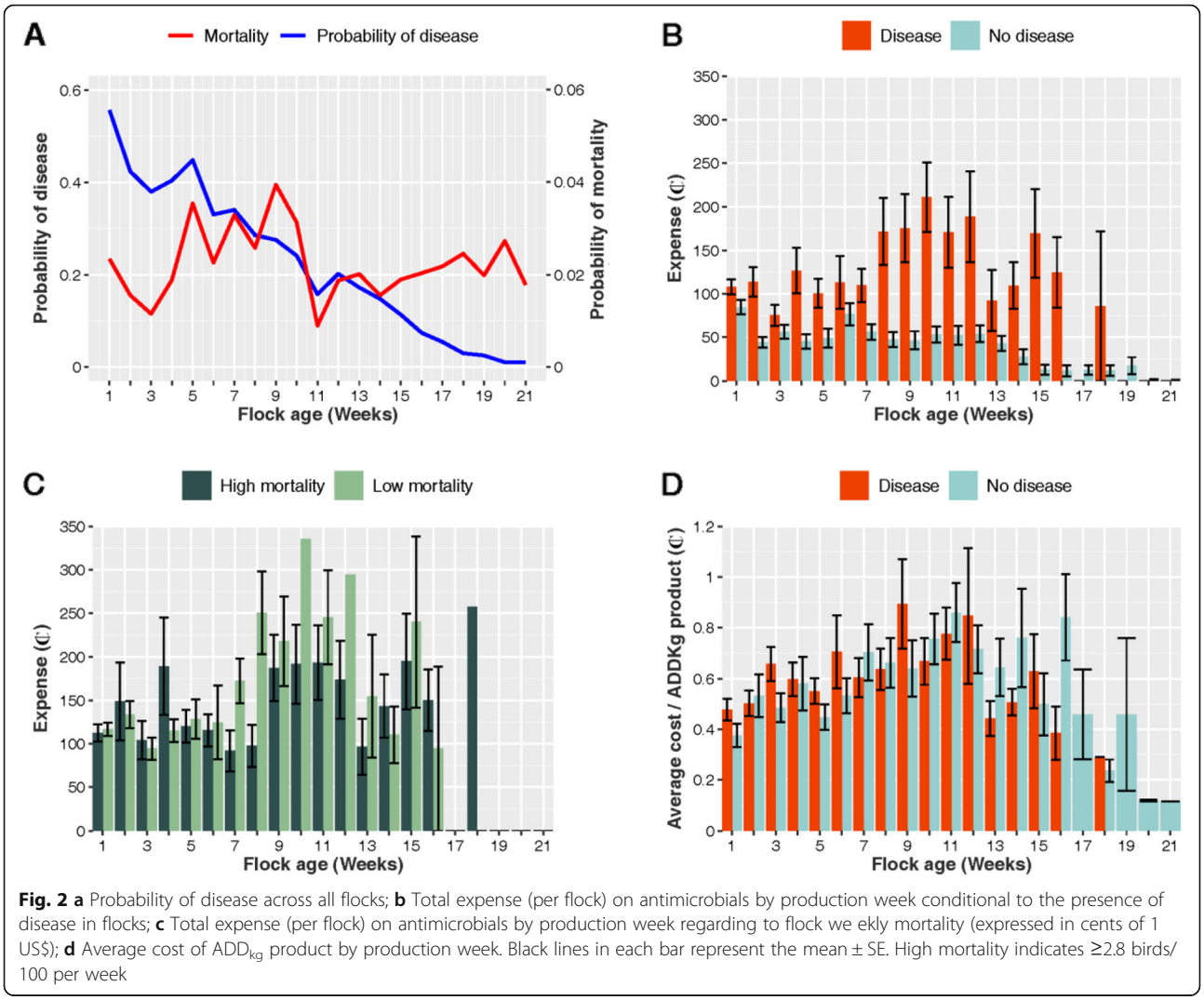

Fig. 2 **a** Probability of disease across all flocks; **b** Total expense (per flock) on antimicrobials by production week conditional to the presence of disease in flocks; **c** Total expense (per flock) on antimicrobials by production week regarding to flock we ekly mortality (expressed in cents of 1 US$); **d** Average cost of ADD_{kg} product by production week. Black lines in each bar represent the mean ± SE. High mortality indicates ≥2.8 birds/100 per week

high priority AAI; 107 (50%) contained an antimicrobial of high importance, and 19 (8.9%) at least one antimicrobial of any other type.

The average farm-adjusted retail price of products (expressed as a daily dose of an antimicrobial product administered to a 1 kg bird, or 1 ADD_{kg}) was ¢0.40 (SE ± 0.05). The retail price (per ADD_{kg} of product), from more to less affordable, corresponded to antimicrobial products containing: (1) exclusively high priority, critically important AAIs (mean ¢0.31 SE ± 0.04 per ADD_{kg}); (2) highest priority, critically important in combination with highly important AAIs (¢0.36; SE ± 0.03); (3) highly important AAIs in combination with other types (mean ¢0.84; SE ± 0.42); and (4) high priority, critically important in combination with other AAIs (mean ¢0.86; SE ± 0.18). With regards to products containing one AAIs, the mean retail price per ADD_{kg} ranged from ¢0.16 (SE ± 0.03) (lincomycin) to ¢5.44 (sulphathiazole) (average price ¢0.48; SE ± 0.03). With regards to products containing two AAIs, the average retail price (per ADD_{kg}) corresponding to each of the AAIs contained was ¢0.21 (SE ± 0.03) ranging from ¢0.03 (SE ± nc) (sulfadiazine) to ¢0.58 (SE ±0.16) (apramycin) (Fig. 3).

Frequency of use and price of antimicrobial products

There was no significant correlation between the frequency of use (number of weeks each product was used on flocks) and the average price of each antimicrobial product (Spearman rank $\rho = 0.05$; $p = 0.495$).

Association between product- and farm-related factors and retail price of products

Three factors were independently associated with a higher retail price of antimicrobial-containing products: (1) Those including AAIs only ($p = 0.007$); (2) Cao Lanh district (compared with Thap Muoi) ($p < 0.001$); and (3) Type of antimicrobial. We evaluated pair-wise differences between all four categories of antimicrobials, and

Table 1 Classification of 214 antimicrobial-containing products based on their AAI composition and WHO classification, as well as their frequency of use and retail price (based on 775 farmer pricing records)

WHO category	No. products ($N = 214$) (%)	AAIs in products (No. of products in bracket)	No. farms using ($N = 100$) (%)	No. flocks using ($N = 191$) (%)	Mean price per product ADD_{kg} (¢) (±SE)
Highest priority+High priority	43 (20.1)	colistin+amoxicillin (12), colistin+ampicillin (12), colistin+neomycin (8), colistin+gentamicin (2), colistin+apramycin (1), tylosin+gentamicin (5), tylosin+amoxicillin (2), tylosin+streptomycin (1)	65 (65.0%)	103 (53.9%)	0.52 (±0.03)
Highest priority only	41 (19.2)	enrofloxacin (12), flumequine (9), tilmicosin (6), erythromycin (2), norfloxacin (2), tylosin (1), colistin (2), colistin+tylosin (3), colistin+spiramycin (2), colistin+enrofloxacin (1), colistin+erythromycin (1)	52 (52.0%)	85 (44.5%)	0.45 (±0.07)
Highest priority +Highly important	38 (17.8)	colistin+oxytetracycline (8), colistin+doxycycline (1), colistin+lincomycin (1), colistin+sulfadimethoxine (1), doxycycline+tylosin (6), doxycyline+tilmicosin (1), erythromycin+sulphamethoxazole (2), erythromycin +oxytetracycline (1), kitasamycin+thiamphenicol (1), oxytetracycline +neomycine (2), oxytetracycline+ spiramycin (3), oxytetracycline+tylosin (2), tylosin+ sulfadimidine (3), tylosin+tetracycline (2), tylosin+sulfachloropyridazine (1), tylosin+ sulfamethazine (1), tylosin+sulphamethoxazole (1)	88 (88.0%)	163 (85.3%)	0.36 (±0.03)
Highly important only	35 (16.4)	oxytetracycline (9), oxytetracycline+sulfadimidine (1), oxytetracycline+thiamphenicol (1), doxycycline (5), doxycycline+florfenicol (3), doxycycline+lincomycin (1), florfenicol (8), lincomycin (2), cephalexin (1), cefadroxil (1), sulphathiazole (1), sulfamethoxypyridazine +tetracycline (1), sulphamethoxazole+ thiamphenicol (1)	58 (58.0%)	77 (40.3%)	0.46 (±0.06)
High priority+Highly important	25 (11.7)	lincomycin+spectinomycin (6), doxycycline+gentamicin (5), doxycycline+neomycin (1), doxycycline+ampicillin (1), oxytetracycline+streptomycin (5), oxytetracycline+neomycin (4), ampicillin+sulfadimethoxine (1), gentamicin+sulfadimidine (1), streptomycin+sulphamethoxazole (1)	43(43.0%)	63 (33.0%)	0.45 (±0.07)
High priority only	12 (5.6)	amoxicillin (7), neomycin (3), ampicillin (1), streptomycin (1)	19 (19.0%)	23 (12.0%)	0.31 (±0.04)
Highly important +Other	8 (3.7)	sulfadimethoxine+trimethoprim (4), sulfadiazine+ trimethoprim (1), sulfadimidine+trimethoprim (1), sulphamethoxazole+trimethoprim (1), doxycycline+ tiamulin (1)	14 (14.0%)	14 (7.3%)	0.84 (±0.42)
Highest priority +Other	6 (2.8)	colistin+trimethoprim (3), josamycin+trimethoprim (1), spiramycin+trimethoprim (1), colistin+enramycin (1)	14 (14.0%)	14 (7.3%)	0.46 (±0.09)
Other only	3 (1.4)	trimethoprim (2), methenamine (1)	23 (23.0%)	32 (16.8%)	0.57 (±0.04)
High priority +Other	2 (0.9)	gentamicin +trimethoprim (1), neomycin+trimethoprim (1)	6 (6.0%)	6 (3.1%)	0.86 (±0.18)
Highest priority +Highly important+Other	1 (0.5)	tylosin+trimethoprim+sulfadiazine+sulphamethoxazole (1)	1 (1.0%)	1 (0.5%)	0.67 (nc)

only differences between the high priority (the most expensive) and highly important (the least expensive) categories remained significantly different ($p = 0.034$) (Table 2).

Data corresponding to 904 weeks when AMU was reported were used to investigate farm-related factors associated with price (standardized per ADD_{kg}) of antimicrobial product used. These data are shown separately (see Additional file 4). Only two factors remained significant in multi-variable model: (1) age of flock (higher ADD_{kg} retail price in older flocks) ($p < 0.001$) and (2) Cao Lanh district ($p < 0.001$) (Table 3).

Discussion

To our knowledge, this is the first study reporting pricing of antimicrobials intended for veterinary use. Since the study is based on a large random sample of farms and a large number of products, we believe that these results accurately reflect the types of antimicrobials used and their associated prices in the Mekong Delta region of Vietnam. Much of this area shares a similar agro-ecological, demographic, as well as a similar antimicrobial retail landscape. We describe here a high diversity of AAIs used in poultry with a relatively low retail price (mean product price ¢0.40 per ADD_{kg}), in line with a

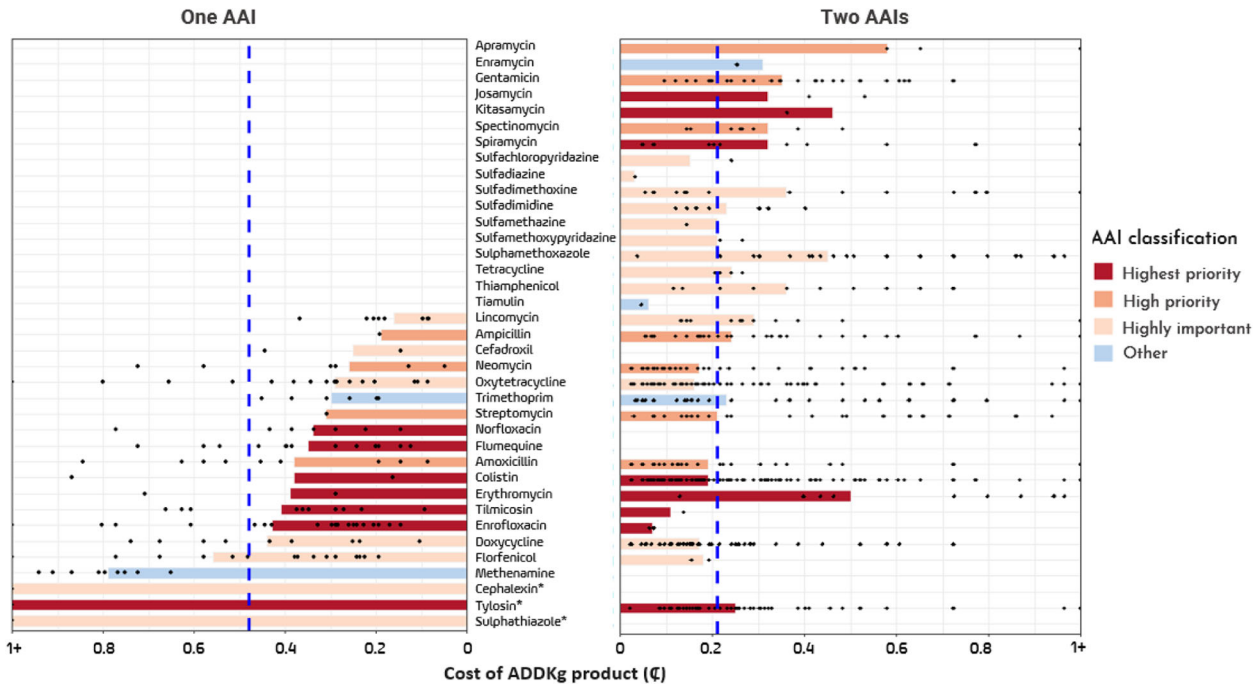

Fig. 3 Average price per ADD_{kg} product stratified by type of AAIs contained. Separate analyses were done from products containing one or two AAIs (*cephalexin ¢2.43; sulphathiazole ¢5.43; tylosin 3.74). The blue dash line indicates the average price per ADD_{kg} (¢) across all products

preliminary study [15]. Overall, retail prices did not greatly differ across WHO classes, with the exception of high priority, critically important antimicrobials, largely driven by the higher prices of apramycin, gentamicin (aminoglycosides) and spectinomycin (aminocyclitol). Over two thirds of the products (68.7%) contained two AAIs. This situation is very different in the European Union where most licenced antimicrobial products contain only one active ingredient [16].

A low retail price of antimicrobial-containing products was not reflected in a higher frequency of use. This is surprising, since often most popular consumer goods and brands tend also to be the most affordable. A study on antimicrobials of human use in Mongolia found that lower-priced antimicrobials were also those purchased more frequently e [17]. This underlines that factors other than retail price drive antimicrobial consumption intended for animal use in the Mekong Delta, and is consistent with the farmers' perception that retail price is not a limiting factor for AMU [18]. Results indicate that the farmers are making judgements on the value of the products when confronted with disease or when treating older flocks that are more valuable. Further research in the area confirmed that farmers chose veterinary drug shops for reasons others than strict pricing, including other services such as advice, diagnostics or even loan services [19].

One of the two study districts (Cao Lanh) was associated with higher retail prices. This district is located

Table 2 Linear random effects models investigating factors associated with the retail price of antimicrobial products. Data corresponding to 775 price measurements corresponding to 213 products containing one or two AAIs were included in the model

	Univariable			Multivariable[a]		
	β	SE	*p*-value	*β*	SE	*p*-value
Two AAIs (baseline = 1 AAI)	0.102	0.060	0.089			
Type of AAI included (baseline = Highly important)						
Highest priority	0.001	0.061	0.993			
High priority	0.141	0.059	0.017	0.122[b]	0.057	0.034
Other	0.111	0.101	0.274			
Pure AAI in product (baseline = mixed with other products)	0.186	0.056	< 0.001	0.152	0.056	0.007
Cao Lanh district (baseline = Thap Muoi)	0.464	0.070	< 0.001	0.468	0.071	< 0.001

[a]Intercept = −1.221 (SE = 0.062); [b]Baseline = All other types of AAIs combined

Table 3 Linear random effects models investigating farm-related factors associated with ADD_{kg} price of antimicrobial products used. Data on 904 price estimates corresponding to weeks when farmers administered antimicrobials were used

	Univariable			Multivariable[a]		
	β	SE	*p*-value	*β*	SE	*p*-value
Farm owner's age (years)(Baseline = < 36)						
36–54	0.197	0.139	0.161			
> 54	0.031	0.156	0.843			
Farm owner's gender (Baseline = Female)						
Male	0.323	0.150	0.033			
Farm owner's experience in raising chickens (years)(Baseline = 0–2)						
> 2–4	− 0.021	0.114	0.853			
> 4	−0.008	0.154	0.958			
Farm owners' education attainment(Baseline = Post high school)						
Primary school	0.514	0.236	0.033			
Secondary school	0.387	0.229	0.096			
High school	0.320	0.235	0.177			
Chicken total (log)	0.061	0.061	0.319			
Age of flock (weeks) (log)	0.157	0.025	< 0.001	0.153	0.024	< 0.001
Disease status (baseline = No disease)						
Disease	0.085	0.050	0.084			
Mortality > 2.8/100 birds/week (Baseline ≤2.8/100 birds/week)	0.091	0.056	0.108			
Cao Lanh district (Baseline = Thap Muoi)	0.533	0.083	< 0.001	0.514	0.081	< 0.001

[a]Intercept = −1.331 (SE = 0.066)

closer to the provincial capital, and has a greater density of veterinary drug shops than the other study district. This difference confirms the existence of the variability in terms of market structure across districts. A recent study also showed a higher frequency of AMU in farms located in this district [20], suggesting that demand for these products could be partly responsible for higher prices. This observation, however, does raise the question of whether marginal changes in price will reduce AMU in small-scale livestock systems.

Regardless of the AAI contained, antimicrobial products that contained pure AAIs were more expensive than AAIs mixed with other substances. These products were generally imported and likely to be more expensive. Details on the compounding and wholesale of AAIs requires further investigation.

Notably, in terms of frequency and expressed per kg of live animal, AMU was highest during the first week of the life of flocks. However, the greatest overall expense corresponded to the 8–12 week period, where the incidence of mortality was highest and birds had reached a higher bodyweight. This is consistent with a previous study when mortality was highest during the same period [20]. The older age of the flock was also associated with the choice of more expensive antimicrobial products. These results indicate that farmers are sensitive to the antimicrobial products' cost in relation to their perceived potential effectiveness. Therefore, farmers use more expensive antimicrobials in the face of disease threat, as well as to protect the higher value of older birds. A previous study indicated that giving antimicrobials to chicken flocks made farmers feel more secure, since more expensive antimicrobials are also perceived to be more effective [18].

The study provides conclusive evidence that prices of antimicrobial-containing products used in chicken production systems in the Mekong Delta are extremely low. Even though many of these products contained AAIs of high importance to human medicine, this is not reflected in the retail price. Retail price is only a component of cost for accessing antimicrobials, the other relates to travel to retail sites and barriers restricting their purchase. In the study area, access to retail points is relatively easy, with farmers located on average ~ 2 km from their closest veterinary drug shop [19]; once there the purchase involves a simple request for the product of preference or a consultation describing the flock health and the needs. There is currently no need for prescription (i.e. veterinary fee), therefore the retail cost is an accurate reflection of the actual cost to the farmer of using antimicrobials in their livestock production system.

It has been stated that overuse of antimicrobials and antimicrobial resistance is partly the result of a dysfunctional health system, and that antimicrobial stewardship requires long-term commitment to healthcare provision

[21]. Policies need to strike a balance between access to antimicrobials by those that really need them whilst preventing unnecessary use [22]. A potential measure that may reduce excessive AMU includes the compulsory requirement of a prescription for purchasing antimicrobials intended for veterinary use similar to that currently in place in some developed countries [23]. However, given the large size of the farming community and the limitations of the veterinary services in Vietnam, such policy would be difficult to implement in the short- to mid-term. Levying a tax on antimicrobial products intended for animal use has been suggested as a policy intervention with a potential to reduce excessive AMU [24]. In view of these results, we consider that levying taxes on the most critical important antimicrobial categories would be reasonable policy intervention.

Conclusions

Our study provides conclusive evidence of the comparatively low prices of antimicrobial-containing products used in chicken production systems in the Mekong Delta of Vietnam, and their lack of relatedness with their human medicine relevance. Implementing pricing mechanisms that provide a signal to retailers and farmers and that the products they are selling and using (antimicrobials) are of importance to society is a policy measure worth exploring. We recommend that retail surveys of antimicrobials should be conducted in other areas within Vietnam as well as other countries in region, so that large-scale pricing policy interventions may be implemented. Any changes in pricing policies would require careful monitoring of the demand response of retailers and farmers whilst ensuring lack of adverse effect on animal health. Such work would provide a true basis for evidence-based policy on the pricing of antimicrobial-containing veterinary products.

Materials and methods

Study flocks and data collection

The study was conducted in Dong Thap province (Mekong Delta). Farmers were randomly chosen from the census (2015) of chicken farmers of two districts (Cao Lanh and Thap Muoi). All flocks investigated corresponded to the baseline (i.e. observational) phase of an intervention study. The aim was to recruit 120 farms raising 100–2000 chickens per cycle [25]. There were 13, 264 and 5371 registered chicken farms in Cao Lanh and Thap Muoi districts. According to this census, 275 (Cao Lanh) and 201 (Thap Muoi) farms had a capacity of 100–2000 chickens. A total of 207 farmers raising > 100 chickens according to the 2015 census were randomly chosen and were contacted by letter by the veterinary authorities (sub-Department of Animal Health and Production of Dong Thap, SDAHP). A meeting was held with 199 attending farmers (96%), in which the project aims and methods were introduced. Farmers were asked to contact the project team should they wish to restock within the following 6 months. A total of 102 (51.3%) such farmers restocking with 100–2000 chickens contacted the project team within 6 months of the meeting and expressed their willingness of being enrolled in the study. Each participating farmer was given a purposefully designed diary alongside a large plastic container. Farmers were asked to weekly record in the diary information on the number of chickens, presence of disease and the amounts of any health-related products used, their costs, and the route of administration (oral-water, oral-feed, injectable) in the diaries. They were also asked to keep all containers of any health-related product in the plastic container, as well as the receipts reflecting the purchases of these products. A research team visited each farm on four different times during the duration of each flock production cycle (typically 3–5 months). On the day of the visit, information on the antimicrobial products recorded in the diaries was compiled, and pictures were taken of the products' labels. The data were subsequently uploaded onto a central database. The pictures of the labels of all health-related products administered to the flocks were carefully examined to determine which products contained AAIs, their strength and the mode of administration. Recruited farms were investigated from October 2016 to March 2018.

Data analyses

Retail prices paid by farmers to purchase antimicrobial products for oral administration were compiled. The retail prices of each product was standardized to 'amount required to treat one kg of live chicken' (ADD_{kg}). This was calculated based on the manufacturers' guidelines on product preparation for therapeutic purposes (dilution of the product in water and/or feed), the retail costs of the product (from farmer's diaries), and the estimated 'daily intake' of a 1 kg chicken (estimated in 225 ml water or 63 g feed). For products with an indication for both water and feed preparation, indications for dilution in water were followed. Prices were expressed in cents of 1 US$ (¢), based on an exchange rate of 1 US$ = 23,319 VND (as of 23rd September 2018).

$$\text{Price of Product ADD}_{kg}\ (¢) = \frac{Retail\ price\ of\ product\ (¢)}{\frac{Contents\ (g\ or\ ml)\ x\ DF}{Daily\ feed\ or\ water\ intake\ (g\ or\ ml)}}$$

DF = Dilution factor (volume or weight of antimicrobial product related to volume or weigh of water or feed)

The probability of AMU by week was calculated by dividing the number of flocks using antimicrobials by the number of flocks observed across all weeks. The

total expense on antimicrobial products over the production cycle was calculated by week from usage data, and was related to the number and weight of birds, as well as the presence/absence of disease in the flock. The weight of birds in flocks by week was estimated from a previous study [1]. We compared the farmers' expense on antimicrobials in weeks with and without disease, stratified by level of flock mortality (computed by the number of dead birds divided by the total birds at the beginning of that week). The average retail price of chosen antimicrobial products (expressed in ADD_{kg}) was computed stratified by flock age and disease status. Comparisons between retail prices of antimicrobials used at different ages and between flocks with and without disease were performed using the Kruskal-Wallis statistic.

The correlation between frequency of use and the average price of each antimicrobial product (standardized as ADD_{kg}) was investigated using the Spearman rank correlation coefficient. The frequency of use was expressed as the number of weeks using of each antimicrobial containing products was used.

The price associated with each specific AAI contained in antimicrobial products was expressed in relation to 1 kg of chicken treated with the product (ADD_{kg}). These were calculated by dividing the price of the product by 1, 2 or 4, depending on the number of AAIs included.

Antimicrobial products were then classified by their AAI composition according to the WHO criteria: (1) 'Highest priority, critical important', (2) 'High priority, critical important', (3) 'Highly important', and (4) 'Other'.

The potential association between product-related factors and their retail price to the farmer (expressed as ADD_{kg} product) was investigated by building a random effect multivariable linear model with 'Farm' specified as a random effect. Factors investigated as fixed-effect covariates were: (1) Number of AAIs in the product (one or two); (2) Type of AAIs based on WHO classification; (3) Product composition ('only AAIs' or 'AAIs mixed with other substances' in the product); and (4) farm district location (Thap Muoi, Cao Lanh). We also investigated the association between farm- and farmer-related factors and retail price by building an additional model with he following covariates as fixed effects: (1) Farm owner's age (log); (2) Farm owner's gender; (3) Farm owner's experience in poultry farming (years); (4) Farm owner's highest education attainment; (5) Flock size (number of chickens) (log); (6) Age the flock (weeks) (log); (7) Flock disease status (yes/no); (8) Flock weekly mortality; (9) District location (Cao Lanh/Thap Muoi). A step-wise forward approach was followed in model building. First univariable models were built, and variables with an associated $p < 0.20$ were screened for multivariable analyses. Only variables with $p \leq 0.05$ were retained in the final multivariable model. Final model residuals were checked for normality and outliers were excluded in a subsequent analysis. All analyses were carried out using R software (version 3.4.3) with the 'lme4' and 'lmerTEST" packages.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12992-019-0539-x.

Additional file 1. Description of 214 antimicrobial-containing products. Description of each of 214 antimicrobial-containing products, their AAI content, as well as their price.

Additional file 2. Price estimates of each of antimicrobial-containing products. Price paid by farmers for each purchase of a single antimicrobial-containing product.

Additional file 3. Table summarizing retail costs of one Animal Daily Dose administered to 1 kg chicken (ADDkg) for 213 products containing one or two AAI each.

Additional file 4. Average cost of antimicrobials (ADDkg) used by week in farms. Average cost of ADDkg of antimicrobials given by farms in relation to flock-related variables.

Abbreviations

AAI: Active antimicrobial ingredient; ADD: Animal daily dose; AMU: Antimicrobial usage; CIAs: Critically important antimicrobials; DF: Dilution factor; IQR: Inter-quartile range; LMICs: Low- and middle-income countries; SDAH-DP: Sub-Department of Animal Health and Production of Dong Thap; SE: Standard error; ViParc: Vietnamese Platform for Antimicrobial Reductions in Chicken production; WHO: World Health Organization

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Authors' contributions

NTTD, JCM and BDT conceived and designed the study. HDP, BTK and NVC carried out data collection on drug shops and farms; NTTD, NTBV and CR performed data analyses; VBH NVC and HDP and TKB contributed to data entry, NTTD, JCM, JR and GT contributed to writing up and editing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used in the manuscript are available as additional files.

Ethics approval and consent to participate

This study was granted ethics approval by the Oxford Tropical Research Ethic Committee (OXTREC) (Ref. 5121/16) and by the local authorities (People's Committee of Dong Thap province).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Wellcome Vietnam Africa Asia Program, Oxford University Clinical Research Unit, 764, Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam. [2]InterRisk program, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand. [3]Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Ho Chi Minh City, Vietnam. [4]Sub Department of

Animal Health and Production, Cao Lanh, Vietnam. [5]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand. [6]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK. [7]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, Oxford University, Oxford, UK.

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Publisher's Note

Antimicrobial Resistance and Infection Control

SHORT REPORT Open Access

An estimation of total antimicrobial usage in humans and animals in Vietnam

Juan J. Carrique-Mas[1,2*], Marc Choisy[1,3,4], Nguyen Van Cuong[1], Guy Thwaites[1,2] and Stephen Baker[1,5]

Abstract

The accurate assessment of antimicrobial use (AMU) requires relating quantities of active ingredients (AAIs) with population denominators. These data can be used to prioritize potential sources of selective pressure for antimicrobial resistance and to establish reduction targets. Here, we estimated AMU in Vietnam (human population 93.4 M in 2015), and compared it with European Union (EU) data (population 511.5 M in 2014). We extrapolated AMU data on each key animal species and humans from different published sources to calculate overall AMU (in tonnes) in Vietnam. We then compared these data with published statistics on AMU in the European Union (EU). A total of 3838 t of antimicrobials were used in Vietnam, of which 2751 (71.7%) corresponded to animal use, and the remainder (1086 t; 28.3%) to human AMU. This equates to 261.7 mg and 247.3 mg per kg of human and animal biomass, compared with 122.0 mg and 151.5 mg in the EU. The greatest quantities of antimicrobials (in decreasing order) were used in pigs (41.7% of total use), humans (28.3%), aquaculture (21.9%) and chickens (4.8%). Combined AMU in other species accounted for < 1.5%. These results are approximate and highlight the need to conduct targeted surveys to improve country-level estimates of AMU.

Keywords: Antimicrobial use, Surveillance, Human medicine, Veterinary medicine, Vietnam, European Union

Main text

Antimicrobial resistance (AMR) in bacterial pathogens is now firmly recognized as major global health problem [1]. AMR arises as a direct consequence of antimicrobial usage (AMU) in humans and animals and resistant organisms and AMR-encoding genes are capable of crossing species barriers [2]. Therefore, the emergence and transfer of AMR means that control solutions need to be conducted from a 'One Health' perspective [3]. However, if we are to reduce AMR we need accurate estimates of where the majority of AMU occurs. Sustained surveillance and monitoring of AMU are widely acknowledged as critical components of the fight against AMR and one of the strategic priorities of the AMR Global Action Plan (GAP) [4].

There is considerable uncertainty regarding AMU in different animal species and humans in most countries. This knowledge gap is due to the absence of reliable AMU data in humans and animals and ill-defined animal population denominators. Many higher income countries, such as those within the European Union (EU), regularly publish their data on AMU in humans and animals, and relate these values to denominator populations in terms of biomass [5]. Conversely, the majority of low- and middle-income countries (LMICs) do not regularly collect and report equivalent AMU statistics.

Recently, the World Organization for Animal Health (OIE) estimated that worldwide, on average 168.7 mg of antimicrobial active ingredients (AAIs) were used to raise 1 kg of animal biomass [6]. Although the report does not include between-country- or species-specific data, it shows however considerable differences between different OIE regions. However, this report did not indicate which animal production sectors are responsible for the largest degree of AMU. Such data are essential for estimating where AMR is most likely to be generated and maintained and pivotal for policy makers to set reduction targets. Here, by integrating various data sources, we aimed to estimate AMU in humans and different animal populations in Vietnam. These data were

* Correspondence: jcarrique-mas@oucru.org
[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam
[2]Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, Oxford University Clinical Research Unit, 764 Vo Van Kiet, Ward 1, District 5, Ho Chi Minh City, Vietnam
Full list of author information is available at the end of the article

compared against available human and animal AMU statistics from the EU.

Human biomass in Vietnam was calculated using 2015 population data stratified by age [7]. Adult (> 18 years-old) body weight was taken from published Figs. (58.4 kg males; 50.8 kg females) [8]. For non-adult age-gender strata, we assigned bodyweights to US populations [9], after adjusting for the difference in body mass between populations in the two countries. This was achieved by applying the correction factors of 0.642 and 0.651, which represent, respectively, the ratios of weights of adult males and adult females in the two countries. The total biomass of terrestrial animals in Vietnam was calculated from official statistics [10] following the approach used by the OIE [6] that combined data on the number of slaughtered animals and standing populations. For aquaculture (farmed fish and shellfish), production data broken down by type of market (domestic, export) (2016) were used [11].

Data on human AMU in Vietnam were extracted from a multi-country survey in hospitals and the community [12]. The reported number of Defined Daily Doses (DDD) (per 1000) were converted to weight of antimicrobial active ingredient (AAI) using the four most common administered antimicrobials (ceftriaxone, ampicillin, azithromycin and levofloxacin). The daily consumption data was extrapolated for a whole year (365 days).

For pigs, chickens, and aquaculture (all aquatic species combined) data on AMU were obtained from quantitative published surveys [13–16]. Data on on AMU through consumption of commercial feed (i.e. antimicrobial growth promoters) were extrapolated from a survey of 1462 pig and chicken commercial feeds in Vietnam [17]. Antimicrobial consumption in aquaculture was extrapolated from a previous study [18], assuming that, on average, antimicrobial products have a 20% strength (weight of AAI related to total weight of product) based on the same study. For ruminants (bovines, buffaloes, sheep, goats) data on AMU in Japan (a high-income country in Asia) for 2010 were used [19]. For non-chicken poultry species (ducks, Muscovy ducks, geese and quails) the authors could not find any published data. AMU was, therefore, conservatively estimated as 50% of that reported in chickens, based on the authors' field experience. We excluded companion animals and equines since no AMU data are available. Best and worst-case AMU scenarios (i.e. lowest and highest AMU) were calculated for all species: for poultry species, upper and lower limits were calculated based on ±25% of the final AMU estimate. For ruminants, the lower limit of AMU was taken from Japanese cattle AMU statistics [19]. The upper limit was set at 50% higher than this estimate; for our summary estimations we used the intermediate value between these two limits. We compared the resulting AMU data with those published in the second ECDC/EFSA/EMA Joint Report on AMU (data for 2014), corresponding with AMU data in relation to the total biomass of terrestrial animal species in 28 EU countries [5] as well as with the Third World Organization for Animal Health (OIE) Report [6] (data for 2015).

Our estimates of human and animal biomass in Vietnam from the above calculations are 4153 and 11, 125 thousand tonnes, respectively (Table 2 in Appendix 1 and Table 1). Estimates of AMU showed that in 2015, a total of 3842 t of antimicrobials were used in Vietnam, of which 2751 (71.7%) was associated with animal use, and the remainder (1086 t; 28.3%) corresponded to human AMU. The greatest quantities of antimicrobials (in decreasing order) were used in pigs (41.7% of total use), humans (28.3%), aquaculture (21.9%) and chickens (4.8%). Combined AMU in other species accounted for < 1.5% (Table 1 and Fig. 1). We estimate that, in total, 261.7 mg (131.4–394.3 mg) of AAI were administered per 1 kg of human and 247.3 mg (130.3–364.3 mg) per 1 kg of animal in Vietnam. The corresponding figures from the EU were 122.0 mg/kg and 151.1 mg/kg in humans and animals, respectively (Fig. 2).

Here, using a combination of available statistics alongside published AMU survey and extrapolation data, we estimated AMU related to biomass in humans and animal production in Vietnam. Our results suggest that in this country pig production and aquaculture should be the main target if the country aims to reduce its AMU footprint in animal production. AMU in humans in Vietnam (32.0 DDD per 1000 inhabitants per day) ranks higher than in most countries in the EU. These human data were generated using limited retail surveys [12]. However, EU countries such as Romania, Greece, France, Spain, and Ireland featured a higher magnitude of AMU (in terms of DDD related to population) than Vietnam. A recent report from Thailand, a LMIC country which is more comparable to Vietnam, estimated that in 2017 a total of 53.0 DDD per 1000 inhabitants per day were used in 2017 [20]. The Thai study used surveillance data on declared quantities of antimicrobials, which is a compulsory requirement for companies trading with antimicrobials in that country.

Whilst these are the first specific calculations for AMU in Vietnam, there is a considerable uncertainty around these estimates due to the lack of reliable data. For example, AMU data in humans, pigs, and aquaculture originate from single studies, all conducted prior to 2015. Furthermore, there are no data whatsoever on AMU in non-chicken poultry species and ruminants. The situation is likely to be even

Table 1 Calculation of total annual AMU in each animal production type

Category	Sub-category	No. of animals	Type of data[a]	Weight unit (kg)	Annual bodymass (kg)	AMU[b] (mg per kg)	AGPs in commercial feed (mg per kg)	Total AMU (mg per kg)	Total AMU (tonnes)
Swine	Breeding pigs	4,128,032	Census	240	990,727,726	46.1[1]	286.6[2]	332.7	329.6
	Slaughter pigs (except breeders)	48,567,582	Production	78.6	3,817,411,914	46.1[1]	286.6[2]	332.7	1270.1
Poultry	Chickens	88,777,000	Production	1.8	699,798,600	187.7[3, 4]	77.4[2]	265.1	185.5
	Ducks	101,931,884	Production	2	203,863,767	93.9[5]	38.7[5]	132.6	27.0
	Muscovies	17,652,638	Production	3.2	56,488,440	93.9[5]	38.7[5]	132.6	7.5
	Geese	641,212	Production	3.2	2,051,877	93.9[5]	38.7[5]	132.6	0.3
	Quails	13,526,147	Production	0.13	1,758,399	93.9[5]	38.7[5]	132.6	0.2
Bovine	Breeding bovines	3,472,891	Census	325	1,128,330,008	52.4[6]	0.0	52.4	59.1
	Slaughter bovines (except breeding animals)	1,220,131	Production	200	244,026,240	52.4[6]	0.0	52.4	12.8
Buffalo	Breeding buffaloes	378,549	Population	500	189,274,500	52.4[6]	0.0	52.4	9.9
	Slaughter buffaloes	297,216	Production	300	89,164,711	52.4[6]	0.0	52.4	4.7
Sheep	Breeding animals (est.)	26,901	Census	75	2,017,556	52.4[6]	0.0	52.4	0.1
	Number slaughtered (except breeders)	64,368	Production	75	4,827,600	52.4[6]	0.0	52.4	0.3
Goats	Breeding animals (est.)	444,411	Census	75	33,330,833	52.4[6]	0.0	52.4	1.7
	Number slaughtered (except breeders)	699,515	Production	75	52,463,597	52.4[6]	0.0	52.4	2.7
Aquaculture	All species (domestic)	–	Production		835,000,000	477.1[7]	–	477.1[7]	398.5
	All species (export)	–	Production		2,775,000,000	159.1[8]	–	159.1[8]	441.4
	All animals				11,125,535,768				2751.4

AMU Antimicrobial use, *AGPs* Antimicrobial growth promoters (in commercial feed)

[a]Data derived from official country statistics [10, 11]. 'Census' refers to 'No. standing animals', 'Production' refers to 'No. of slaughtered animals', except for aquaculture, where it refers to 'No. of kg produced'

[b]Excluding antimicrobial growth promoters in commercial feed; [1] Nguyen et al. (2016) [15]; [2] Van Cuong et al. (2016) [17]; [3,4] Average of two studies: Carrique-Mas et al. (2014) [13] and Cuong et al. (2019) [14]; [5] Based on 50% of quantities used in chicken production; [6] Hosoi et al. (2014) [19]; [7] Pham et al. (2015) [16]; [8] Assuming that AMU for export production is 1/3 of the magnitude of AMU for domestic production

worse in other LMICs where there are practically no AMU data in any production sector.

Since different animal types are raised over variable periods, the same magnitude of AMU related to body mass may have different implications for the development and maintenance of AMR For example, in Vietnam chickens are raised over a period ranging from 1 to 5 months, compared with 5–8 months for pigs. The implications of this need to be further investigated.

Because of its relative simplicity, we propose to regularly (i.e. annually) estimate/update quantities of antimicrobials used in relation to body mass as a first step to develop a fully-fledged AMU surveillance system. These estimates could be fine-tuned by conducting targeted surveys tailored to different production types (i.e. meat chickens, layers, breeders, fattening pigs, etc.). It may also be necessary to differentiate the extent of AMU by level of intensification of the production system (i.e. backyard, small-scale, large-scale, industrial), as different systems require variable quantities of antimicrobials. It has been shown that in the Mekong Delta of Vietnam smaller chicken farms tend to use more antimicrobials [13]. Lastly, it would be desirable to incorporate detailed information regarding the classes and formulations of antimicrobials used, since there is a great variability

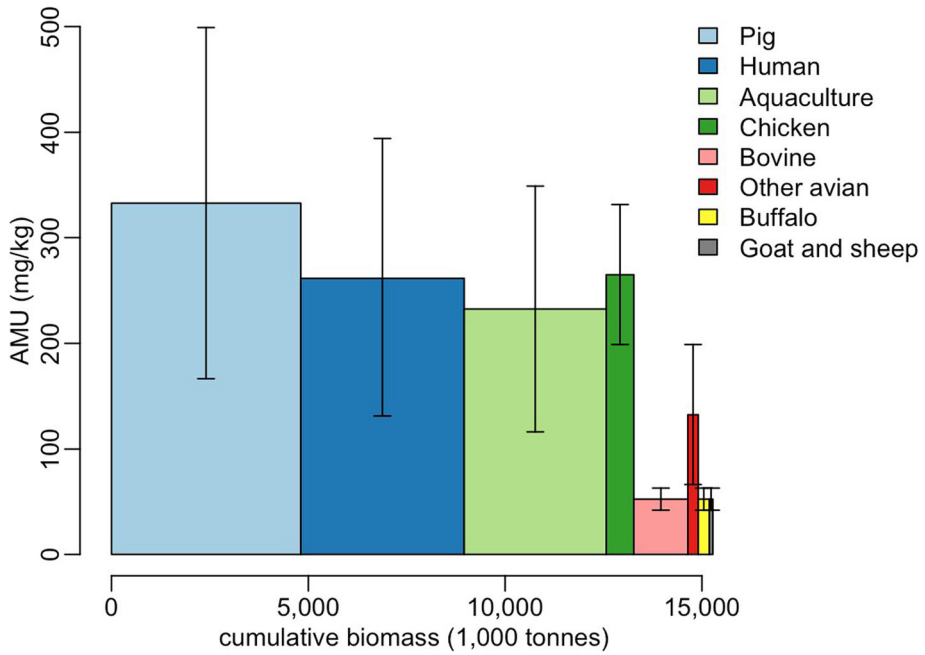

Fig. 1 Two-dimensional diagram representing the estimated annual amounts (areas of bars) of antimicrobials used in each of species (including humans) in Vietnam, and whether these quantities are more affected by the total biomass (width of bars) or the intensity of AMU (height of bars). Bars are sorted from higher to lower overall AMU. AMU = Antimicrobial use. The vertical lines represent the range between best- and worst-case scenarios. 'Other avian' includes ducks, muscovies, geese and quails

regarding the strength of different antimicrobial products and their impact on development of AMR.

In conclusion, in the absence of reliable statistics on sales of AAIs, the challenges of monitoring AMU in animal production in LMICs such as Vietnam can be overcome by the use of innovative approaches that maximize the use of existing animal population statistics and AMU data. These estimates should help elucidate secular changes in AMU and help refine policies and interventions aimed at reducing AMU at country level.

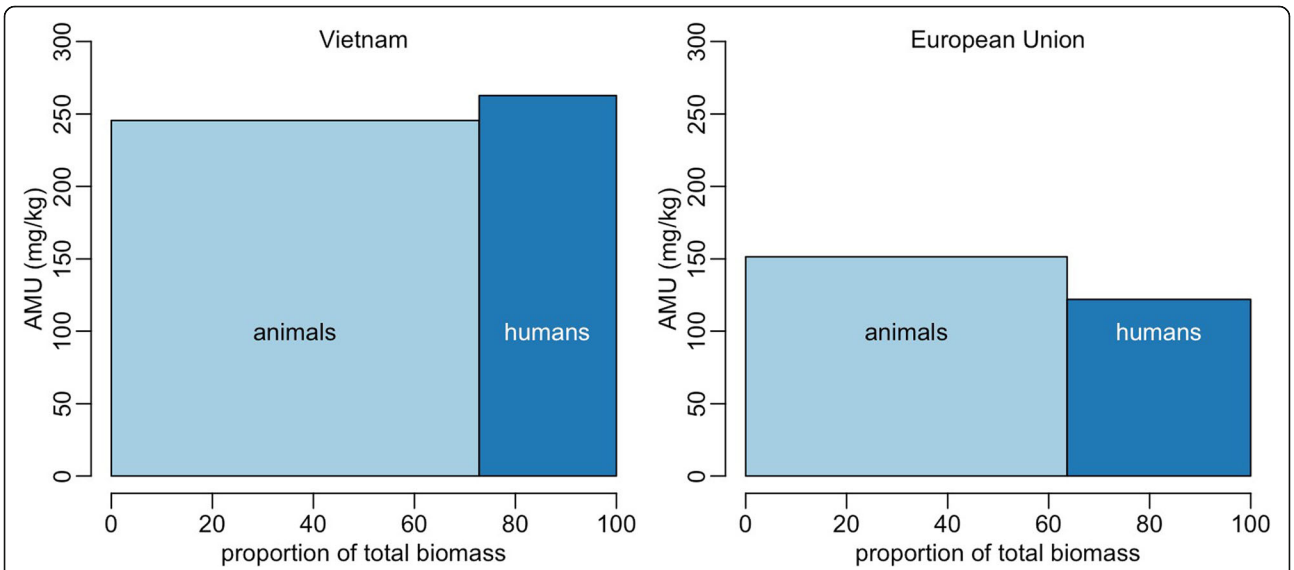

Fig. 2 Two-dimensional diagram showing the relative annual amounts of AMU in the European Union (2014) and in Vietnam. In order to render comparison between European Union and Vietnam possible, the biomasses of animals and humans on the *x*-axis have been scaled to proportions. AMU = Antimicrobial use

Appendix 1

Table 2

Table 2 Estimation of human bodymass of the Vietnamese population from population pyramid, adult bodyweight and age-gender specific bodyweights

Age (years)	Total population			Average weight (US) (kg)		Average weight (Vietnam)[a] (kg)		Estimated bodymass (kg)		
	Males	Females	Total	Males	Females	Males	Females	Males	Females	Total
> 18	32,448,992	32,448,992	64,897,984			58.4	50.8	1,895,021,133	1,648,408,794	3,543,429,926
15 to 18	3,582,536	3,390,322	6,972,858	64.4	54.4	41.4	35.4	148,234,592	120,066,219	268,300,812
10 to 14	3,406,698	3,207,976	6,614,674	39.9	41.5	25.6	27.0	87,333,258	86,668,284	174,001,542
5 to 9	3,774,596	3,446,644	7,221,240	22.9	22.4	14.7	14.6	55,536,575	50,260,341	105,796,916
0 to 4	4,078,564	3,662,281	7,740,845	12.5	12.0	8.0	7.8	32,755,967	28,609,739	61,365,706
			93,447,601					2,218,881,525	1,934,013,377	4,152,894,902

[a]Estimated from US data after applying a correction factor 0.642 (males) and 0.651 (females)

Appendix 2

Table 3.

Table 3 Estimation of weight of antimicrobial active ingredient from antimicrobials consumed by humans

Antimicrobial class	Antimicrobial	No. DDDs (per 1000 inhabitants per day)	Dose in g (for a typical inhabitant)	Daily amount of AAI (per 1000 inhabitants) (g)
Cephalosporin	Ceftriaxone	8	1.5	12
Broad-spectrum beta lactam	Ampicillin	8	1.5	12
Macrolide	Azithromycin	8	0.5	4
Oral fluoroquinolone	Levofloxacin	8	0.5	4
All				32

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Authors' contributions
SB and JC conceived the study and wrote the first draft of manuscript. MC and NC contributed to data analysis and drawing the figures. GT contributed to the discussion. All authors contributed to final version. All authors read and approved the final manuscript.
DDDs Defined Daily Doses, *AAI* Antimicrobial active ingredient

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Availability of data and materials
The data presented and analysed here have all been extracted from publicly available data sets and publications cited in the body text.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam. [2]Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, Oxford University Clinical Research Unit, 764 Vo Van Kiet, Ward 1, District 5, Ho Chi Minh City, Vietnam. [3]MIVEGEC, IRD, CNRS, University of Montpellier, Montpellier, France. [4]LMI "Drug Resistance in South-east Asia" (DRISA), Hanoi, Vietnam. [5]Cambridge Institute of Therapeutic Immunology & Infectious Disease, Department of Medicine, University of Cambridge, Cambridge, UK.

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Communication

Characterizing Antimicrobial Resistance in Chicken Pathogens: A Step towards Improved Antimicrobial Stewardship in Poultry Production in Vietnam

Nguyen Thi Phuong Yen [1], Nguyen Thi Nhung [1], Nguyen Thi Bich Van [1], Nguyen Van Cuong [1], Bach Tuan Kiet [2], Doan Hoang Phu [1,3], Vo Be Hien [2], James Campbell [1,4], Niwat Chansiripornchai [5], Guy E. Thwaites [1,4] and Juan J. Carrique-Mas [1,4,*]

1 Oxford University Clinical Research Unit, Ho Chi Minh 700000, Vietnam; yenntp@oucru.org (N.T.P.Y.); nhungnt@oucru.org (N.T.N.); vanntb@oucru.org (N.T.B.V.); cuongnv@oucru.org (N.V.C.); phudh@oucru.org (D.H.P.); jcampbell@oucru.org (J.C.); gthwaites@oucru.org (G.E.T.)
2 Sub-Department of Animal Health and Production, Dong Thap 81000, Vietnam; bachkiettydt1@gmail.com (B.T.K.); hienthuydt@gmail.com (V.B.H.)
3 Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh 700000, Vietnam
4 Centre for Tropical Medicine and Global Health, Oxford University, Oxford OX3 7FZ, UK
5 Avian Health Research Unit, Chulalongkorn University, Bangkok 10330, Thailand; Niwat.C@chula.ac.th
* Correspondence: jcarrique-mas@oucru.org

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Abstract: In the Mekong Delta of Vietnam, farmers use large quantities of antimicrobials to raise small-scale chicken flocks, often including active ingredients regarded of "critical importance'" by the World Health Organization. Due to limitations in laboratory capacity, the choice of antimicrobials normally does not follow any empirical criteria of effectiveness. The aim of this study was to highlight non-critically important antimicrobials against which chicken pathogens are likely to be susceptible as a basis for treatment guidelines. Microtiter broth dilution method was performed to determine the minimal inhibitory concentration (MIC) of 12 commonly used antimicrobials for 58 isolates, including *Ornithobacterium rhinotracheale* (ORT) (n = 22), *Gallibacterium anatis* (n = 19), and *Avibacterium endocarditidis* (n = 17). Unfortunately, internationally accepted breakpoints for resistance in these organisms do not exist. We drew tentative epidemiological cut-offs (TECOFFs) for those antimicrobial-pathogen combinations where MIC distributions suggested the presence of a distinct non-wild-type population. Based on the observed results, doxycycline would be the drug of choice for *A. endocarditidis* (11.8% presumptive non-wild type) and *G. anatis* infections (5.3% presumptive non-wild type). A total of 13.6% ORT isolates were non-wild type with regards to oxytetracycline, making it the drug of choice against this pathogen. This study illustrates the challenges in interpreting susceptibility testing results and the need to establish internationally accepted breakpoints for veterinary pathogens.

Keywords: antimicrobial resistance; minimal inhibitory concentration; chicken pathogens; bacteria; diseases; Vietnam; low- and middle-income countries

1. Introduction

Antimicrobial resistance (AMR) is a major worldwide health emergency [1]. Much of the concern derives from its impact on human health. It has been estimated that AMR-related infections will reach 10 million cases per year in 2050 [2]. There is a scientific consensus that excessive antimicrobial use (AMU) and AMR in animal populations are contributing factors to global AMR [3]. The issue of AMR in animal pathogens has received much less attention than AMR in human pathogens, and thus there

is a deficit of published surveillance and research data. This is partially due to limited veterinary diagnostic capacity, especially in low and middle-income countries (LMICs) [4]. The presence of AMR traits in animal pathogens is likely to entail considerable, but yet to be quantified, economic losses derived from the failure to treat diseases [5]. Globally, over 110,000 tons of chicken meat are produced each year, making it the second most consumed type of meat in the world. Furthermore, by 2025, chicken meat production is expected to surpass that of pork [6]. A large number of bacterial pathogens can infect chicken flocks, and many such organisms are resistant to commonly used antimicrobials in farms [7]. High levels of disease and mortality are regarded as major drivers of AMU in flocks in the region, and respiratory diseases are among the most prevalent ones [8]. A number of bacterial pathogens, including colisepticaemic *E. coli*, *Avibacterium paragallinarum*, *Ornitobacterium rhinotracheale* (ORT) and *Mycoplasma gallisepticum* were detected in diseased chicken flocks in the Mekong Delta of Vietnam [9]. Previous reports have indicated extremely high levels of AMU in small-scale chicken flocks in the same region, as well as high levels of antimicrobial resistance in commensal *E. coli* of chicken origin [10–12]. However, there are no published data regarding levels of phenotypic resistance in chicken pathogens in flocks in the country. Current scientific consensus indicates that antimicrobials regarded by the World Health Organization (WHO) to be of critical importance for human medicine should be restricted in veterinary medicine [13] and this has recently become integrated in the policy of several countries [14,15]. Using microtiter broth dilution, we characterized the phenotypic resistance of three global chicken bacterial pathogens in the Mekong Delta (Vietnam) to commonly used antimicrobials in the area. The data on the antimicrobial susceptibility of these organisms should form the basis of treatment guidelines that prioritize the choice of antimicrobial classes that do not include critically important antimicrobials according to the WHO [16]. However, widely accepted breakpoints for the interpretation of resistance for most poultry pathogens do not exist. In veterinary medicine, setting clinical breakpoints is challenging given the range of animal species and pathogens involved. Resistance has often been defined in terms of epidemiological cut-offs (ECOFFs). These cut-offs are drawn based on the MIC distributions that have been used to distinguish between wild-type and non-wild-type populations [17]. Based on the minimal inhibitory concentration (MIC) distributions of different antimicrobial-pathogen combinations, we proposed "tentative" epidemiological cut-offs (TECOFFs) for three different poultry pathogens common in the Mekong Delta region of Vietnam. This work is the first step aiming to characterize antimicrobial susceptibility of veterinary pathogens in Vietnam. These results should be the basis of future guidelines to veterinarians and drug shop owners in the country.

2. Results

MIC results are shown in Supplementary Table S1 and are summarized in Table 1 and Figure 1. For 29 (80.5%) antimicrobial-pathogen combinations, we observed a bimodal (n = 18) or multimodal (n = 11) distribution. The lower mode of these suggested a wild-type sub-population, and therefore TECOFFs were proposed. For ORT, TECOFFs could be drawn for 8/12 antimicrobials tested. For four of those antimicrobials (enrofloxacin, tylosin, amoxicillin, doxycycline), the proposed TECOFFs agreed with the cut-off values reported previously [18–20].

Given the observed patterns, and in the absence of susceptibility testing of isolates from a given flock, we would suggest doxycycline as the drug of choice for *A. endocarditidis* infections (11.8% presumptive non-wild type) or *G. anatis* infection (5.3% presumptive non-wild type). For ORT oxytetracycline would be a good choice (13.6% non-wild type). As a second choice we would propose florfenicol (17.6% non-wild type) for *A. endocarditidis* and thiamphenicol (22.7% non-wild type) for ORT (Figure 1).

Table 1. Distribution of minimal inhibitory concentrations (MICs) of 12 antimicrobials commonly used for three chicken pathogens from the Mekong Delta of Vietnam.

		Mic Range (μg)														Type of Distribution
		0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**	
***Ornithobacterium rhinotracheale* (N = 22)**	COL	-	-	0	0	0	0	0	0	0	0	14	86	-	-	Unimodal
	ENR	-	-	14	14	0	0	9	9	23	18	9	5	-	-	Bimodal
	TYL	-	0	14	5	18	32	9	0	18	5	0	0	0	0	Multimodal
	GEN	-	-	-	-	0	0	0	9	9	55	27	0	0	0	Unimodal
	NEO	-	-	-	-	5	0	0	18	5	36	32	5	0	0	Bimodal
	STR	-	-	-	-	0	0	0	9	32	41	18	0	0	0	Unimodal
	AMX	-	-	-	-	14	0	5	27	23	14	18	0	0	0	Multimodal
	FFN	-	-	0	32	64	5	0	0	0	0	0	0	0	0	Unimodal
	THA	-	-	-	0	9	32	23	14	0	0	0	0	23	0	Bimodal
	OXY	-	-	-	0	9	14	27	18	14	5	0	0	14	0	Bimodal
	DOX	-	-	-	0	5	18	23	18	32	5	0	0	0	0	Bimodal
	SXT	-	-	-	0	5	27	32	5	18	14	-	-	-	-	Bimodal
***Gallibacterium anatis* (N = 19)**	COL	-	-	0	0	5	79	11	0	0	0	5	0	-	-	Unimodal
	ENR	-	-	0	5	5	5	5	0	11	16	21	32	-	-	Bimodal
	TYL	-	-	-	-	0	0	0	0	0	5	32	11	16	37	Bimodal
	GEN	-	-	0	5	37	16	5	5	0	16	16	0	-	-	Bimodal
	NEO	-	-	-	-	0	32	5	0	11	11	0	11	5	26	Multimodal
	STR	-	-	-	-	0	0	11	16	0	0	0	5	21	47	Bimodal
	AMX	-	-	-	0	5	0	0	5	32	5	0	11	0	42	Multimodal
	FFN				0	47	0	0	0	0	5	26	16	5	0	Bimodal
	THA	-	-	-	0	11	16	0	0	0	0	0	0	16	58	Bimodal
	OXY	-	-	-	-	0	0	0	0	0	0	5	16	26	53	Unimodal
	DOX	-	-	-	-	0	0	0	32	42	21	0	0	0	5	Bimodal
	SXT	11	0	11	5	0	11	0	0	11	63	-	-	-	-	Multimodal
***Avibacterium endocarditidis* (N = 17)**	COL	-	-	0	0	18	29	41	6	6	0	0	0	-	-	Unimodal
	ENR	-		12	6	0	24	0	12	6	18	12	12	-	-	Multimodal
	TYL	-	-	-	-	0	0	6	0	12	29	6	18	12	18	Multimodal
	GEN	-	-	-	0	12	18	29	18	0	0	6	18	0	0	Bimodal
	NEO	-	-	-	-	0	0	24	24	6	12	29	6	0	0	Bimodal
	STR	-	-	-	-	0	0	0	18	29	6	0	6	6	35	Bimodal
	AMX	-	-	-	-	12	18	12	24	12	12	6	0	0	6	Multimodal
	FFN	-	-	-	-	76	6	0	0	0	18	0	0	0	0	Bimodal
	THA	-	-	-	-	6	12	6	0	0	0	0	0	6	71	Bimodal
	OXY	-	-	-	-	0	0	6	0	0	6	41	41	0	6	Multimodal
	DOX	-	-	-	0	12	0	41	35	0	6	0	6	0	0	Multimodal
	SXT	0	12	6	0	0	29	18	0	12	24	-	-	-	-	Multimodal

Key: COL = colistin, ENR = enrofloxacin, TYL = tylosin, GEN = gentamicin, NEO = neomycin, STR = streptomycin, AMX = amoxicillin, FFN = florfenicol, THA = thiamphenicol, OXY = oxytetracycline, DOX = doxycycline, SXT = co-trimoxazole. NC= Not calculated.

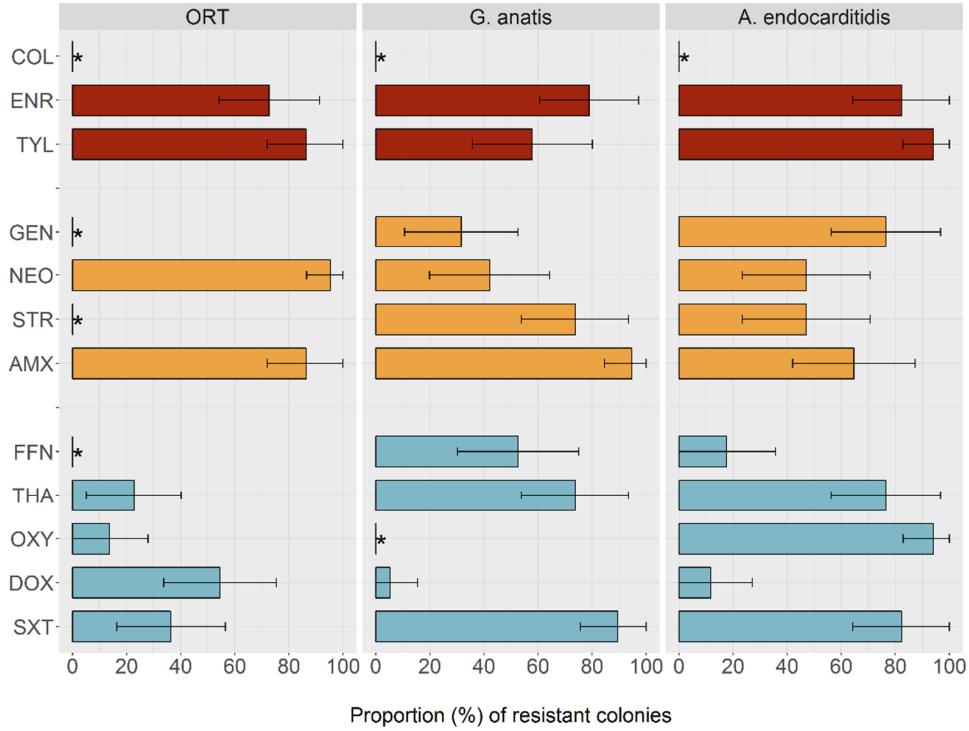

Figure 1. Estimated prevalence of presumptive non-wild phenotypes with regards to 12 antimicrobials among *Ornithobacterium rhinotracheale* (ORT), *G. anatis*, and *A. endocarditidis* isolates from Mekong Delta chicken flocks. Bars indicate percent of isolates that are fully resistant, with 95% binomial confidence intervals drawn around these percentages. Red = highest priority, orange = high priority, blue = highly important antimicrobial according to the WHO. Key: COL = colistin, ENR = enrofloxacin, TYL = tylosin, GEN = gentamicin, NEO = neomycin, STR = streptomycin, AMX = amoxicillin, FFN = florfenicol, THA = thiamphenicol, OXY = oxytetracycline, DOX = doxycycline, SXT = co-trimoxazole. * Tentative epidemiological cut-offs (TECOFFs) could not be established.

3. Discussion

Susceptibility testing of bacterial animal pathogens aims to provide a rational basis for the choice of appropriate antimicrobial therapy. Based on this, the use of non-critically important antimicrobials should be prioritized. In our study, doxyxycline (tetracycline class) is likely to be effective against *A. endocarditidis* and *G. anatis* (11.8% and 5.3% presumptive non-wild types, respectively); thiamphenicol (amphenicol class) is likely to be effective against ORT (22.7% non-wild type), whereas florfenicol (amphenicol class) is likely to be effective against *A. endocarditidis* (17.6% non-wild type). Neither amphenicols nor tetracyclines are classified as critically important antimicrobials by the WHO [16].

For a considerable number (n = 7) of antimicrobial–pathogen combinations, we obtained a unimodal distribution that did not allow TECOFFs to be drawn; further, we observed a multimodal distribution for a relatively high number (n = 11) of combinations. Given the limited number of isolates tested and the uncertainty associated with the chosen interpretative criteria, our results need to be taken with great caution. Data from a larger set of isolates are therefore required to validate these TECOFFs. These results highlight the pressing need to establish internationally accepted interpretation guidelines. As in human medicine, ideally MIC data of antimicrobial–pathogen combinations should be shared across countries, and these should be updated periodically [17]. For colistin, a critically important antimicrobial "of the highest priority" according to WHO widely used in chicken production, interpretation guidelines are restricted to human pathogens [21]. Our data indicate a unimodal distribution for these organisms, and therefore TECOFFs could not be established. Based on the magnitude of the MICs for colistin, it is likely effective against *G. anatis* and, to a lesser extent, *A. endocarditidis*.

Most LMICs have limited capacity for isolating bacterial pathogens and performing antimicrobial susceptibility testing [4]. These deficiencies are particularly severe in veterinary medicine. In Vietnam, diagnostic investigations are seldom carried out in small-scale farming settings due to economic and logistic constraints. Faced with disease, farmers and their advisors often treat flocks with antimicrobials irrespective of the pathogen [5]. A complicating factor is the fact that for many bacterial infections, clinical signs are often non-specific, and mixed infections are common [10].

Since in Vietnam veterinary drug shops are the main points of supply and advice to farmers on AMU [22], results of phenotypic AMR testing of pathogens should be made available to drug shop owners and other animal-health advisors (i.e., commune animal health workers). The study presented here is limited in terms of bacterial species and production types. Therefore, we recommend expanding it to other bacterial pathogens in different production systems. This would require establishing a well-equipped, reference laboratory capable of performing micro-agglutination antimicrobial susceptibility testing and the archiving of isolates. Examination of a (representative) sufficient number of isolates should enable the establishment of reliable ECOFFs. Monitoring changes in MIC distributions over time of commonly used antimicrobials should allow the detection of emerging resistance phenotypes, as well as drafting AMU guidelines aiming at improving the efficacy of antimicrobials used in poultry production whilst preserving those that are critically important antimicrobials for human medicine.

4. Materials and Methods

4.1. Bacterial Isolates

A total of 58 bacterial isolates including *Ornithobacterium rhinotracheale* (ORT) (n = 22), *Gallibacterium anatis* biovar *haemolytica* (n = 19) and *Avibacterium endocarditidis* (n = 17) were investigated. ORT is an emerging respiratory pathogen [23]. *G. anatis* is an opportunistic pathogen that also causes diarrhea, peritionitis, oophoritis [24], as well as systemic infections with high mortality [25] in flocks. *A. endocarditidis* causes vascular as well as hepatic/spleen lesions [26]. All isolates were recovered from diseased chickens that were subjected to a diagnostic necropsy in different locations in Dong Thap province (Mekong Delta). All isolates were recovered at the Sub-Department of Animal Health (Dong Thap) diagnostic laboratory between September 2017 and September 2019. No two isolates came from the same flock. Isolates were recovered using blood agar and chocolate agar (Oxoid, Cheshire, Great Britain) incubated in 5% CO_2 at 35 ± 2 °C for 20–44 h. The species identification of strains was performed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker, Germany). The diagnostic work was carried out under the umbrella of the ViParc project (www.viparc.org). The project was granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Minimal Risk) (Ref. 5121/16).

4.2. Antimicrobial Susceptibility Testing

We investigated the 12 most commonly used antimicrobials in chicken flocks in the area [27], including: colistin (COL), oxytetracycline (OXY), tylosin (TYL), doxycycline (DOX), gentamicin (GEN), amoxicillin (AMX), enrofloxacin (ENR), neomycin (NEO), streptomycin (STR), florfenicol (FFN), thiamphenicol (THA), and co-trimoxazole (SXT). The MIC of these antimicrobials was investigated for study pathogens by broth micro-dilution following Clinical Laboratory Standards Institute (CLSI) procedures outlined in VET01S [28] and M100 [29]. MIC experiments were carried out using cation-adjusted Mueller Hinton-II broth (MHB2, Sigma-Aldrich, St. Louis MO, USA) with 2.5% lysed horse blood (E & O Laboratories, Bonnybridge, UK) in 96-well plates (Corning, Corning, NY, USA). The test ranges for antimicrobials were shown in Table 1. The MICs of bacteria were recorded after 24 h (*G. anatis and A. endocarditidis*) or 48 h (ORT) incubation at 35 ± 2 °C. Reference strains *E. coli* ATCC 25,922 and *Enterococcus faecalis* ATCC 29,212 were used to verify the quality and accuracy of the testing procedures [30].

4.3. Data Analyses

For antimicrobial–pathogen combinations where the MIC followed a distribution suggestive of the existence of wild-type and non-wild type populations, we proposed a tentative epidemiological cut-off (TECOFF) [17]. For antimicrobial–ORT combinations not meeting that criteria, these TECOFFs were compared with those from published studies [18–20]. For each antimicrobial–pathogen combination, we calculated a prevalence of "presumptive non-wild-types" highlighting the antimicrobials not belonging to the WHO critical important classes. Analyses were carried out using R software (www.r-project.org).

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-6382/9/8/499/s1, Table S1: Raw MIC data of all 58 chicken pathogens investigated.

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A novel method for measuring phenotypic colistin resistance in *Escherichia coli* populations from chicken flocks

Nhung Thi Nguyen[a], Nguyen Thi Phuong Yen[a], Nguyen Van Ky Thien[a], Nguyen Van Cuong[a], Bach Tuan Kiet[b], James Campbell[a,c], Guy Thwaites[a,c], Stephen Baker[d], Ronald B. Geskus[a,c], Juan Carrique-Mas[a,c]

[a]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam
[b]Sub-Department of Animal Health and Production, Dong Thap province, Vietnam
[c]Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom
[d]Cambridge Institute of Therapeutic Immunology & Infectious Disease, University of Cambridge, Cambridge, United Kingdom

Running title: Method for measuring colistin resistance in chicken

KEYWORDS

Poultry, colistin resistance, broth microdilution, colistin use.

Corresponding author

Nguyen Thi Nhung
Senior Microbiologist
ViParc Project Laboratory co-ordinator
Oxford University Clinical Research Unit
764 Vo Van Kiet, Ward 1, District 5
Ho Chi Minh City (Vietnam)
E-mail: nhungnt@oucru.org

ABSTRACT

Colistin is extensively used in animal production in many low- and middle-income countries. There is a need to develop methodologies to benchmark and monitor changes in resistance among mixed commensal bacterial populations in farms. We aimed to evaluate the performance of a broth microdilution method based on culturing a pooled *Escherichia coli* suspension (30-50 organisms) obtained from each sample. To confirm the biological basis and sensitivity of the method, we cultured 16 combinations of one colistin-susceptible and one *mcr*-1 encoded colistin-resistant *E. coli* in the presence of 2mg/L colistin. Optical density (OD_{600nm}) readings over time were used to generate a growth curve, and these values were adjusted to the values obtained in the absence of colistin (adjusted Area Under the Curve, AUC_{adj}). The median limit of detection was 1 resistant in 10^4 susceptible colonies [1^{st} - 3^{rd} quartile, 10^2:1 –10^5:1]. We applied this method to 108 pooled faecal samples from 36 chicken flocks from the Mekong Delta (Vietnam), and determined the correlation between this method and the prevalence of colistin resistance in individual colonies harvested from field samples, determined by the Minimum Inhibitory Concentration. The overall prevalence of colistin resistance at sample and isolate level (estimated from the AUC_{adj}) was 38.9% [95%CI, 29.8-48.8%] and 19.4% (SD± 26.3%), respectively. Increased colistin resistance was associated with recent (2 weeks) use of colistin (OR=3.67) and other, non-colistin antimicrobials (OR=1.84). Our method is a sensitive and affordable approach to monitor changes in colistin resistance in *E. coli* populations from faecal samples over time.

IMPORTANCE

Colistin (polymyxin E) is an antimicrobial with poor solubility in agar-based media, and therefore broth microdilution is the only available method for phenotypic resistance. However, estimating colistin resistance in mixed *Escherichia coli* populations is laborious since it requires individual colony isolation, identification and susceptibility testing. We developed a growth-based microdilution method suitable for pooled faecal samples. We validated the method by comparing it with individual MIC of 909 *E. coli* isolates; we then tested 108 pooled faecal samples from 36 healthy chicken flocks collected over their production cycle. A higher level of resistance was seen in flocks recently treated with colistin in water, although the observed generated resistance was short-lived. Our method is affordable, and may potentially be integrated into surveillance systems aiming at estimating the prevalence of resistance at colony level in flocks/herds. Furthermore, it may also be adapted to other complex biological systems, such as farms and abattoirs.

INTRODUCTION

Colistin (polymyxin E) is a last-resort drug used for the treatment of severe multi-drug resistant (MDR) infections in many countries, and currently is classified by the World Health Organization (WHO) as a 'highest priority, critically important' antimicrobial (1). The emergence of *mcr*-1 plasmid-encoded colistin resistance among Gram-negative bacteria is considered a serious threat to global health (2). It has been hypothesized that colistin use in animal production is a major contributing factor to the emergence of colistin resistance worldwide (3). Colistin is still used in poultry and pig farming in many countries (4). In terms of frequency, colistin is the most commonly used antimicrobial in chicken production in the Mekong Delta region of Vietnam (5, 6). Studies in the same region have shown that resistance against colistin in commensal *Escherichia coli* from chicken flocks is often encoded by the *mcr*-1 gene (7, 8). At sample level, the prevalence of *mcr*-1 in chicken faecal samples in the Mekong Delta was 59.4%. The prevalence of this gene has also be found to be higher among in-contact humans (chicken farmers) than in urban individuals (7).

E. coli is an ubiquitous commensal enteric organism globally used to monitor phenotypic antimicrobial resistance (AMR) in national surveillance programmes, both in humans and in animals (9, 10). Given the diversity of this organism within the enteric microbiome, the characterisation of phenotypic resistance in a mixed population of commensal *E. coli* requires selecting a representative and sufficiently large number of strains. This is often achieved by performing differential colony counts on agar media with and without antimicrobials (11). However, agar-based methods are not appropriate for colistin given the antimicrobials' poor diffusion (12). Determination of the minimal inhibitory concentration (MIC) by broth microdilution is regarded as the gold standard for testing of colistin resistance of

Enterobacteriaceae (ISO 20776-1) both by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (12, 13). Establishing accurately the prevalence of resistance at colony level requires the investigation of a sufficiently large, representative number of isolates from each sample, which is extremely laborious and costly (8, 11, 14). Therefore, there is a need for developing cost-effective methodologies for evaluating resistance against colistin in mixed *E. coli* populations from animal faecal samples. Here, we designed and evaluated a broth microdilution-based method to quantify colistin resistance in *E. coli* populations from pooled chicken faecal samples. We then related the observed results to data on antimicrobial use (AMU) from the same flocks.

RESULTS

Growth of standard suspensions

The AUC_{adj} values generated from all susceptible:resistant combinations are presented in Fig.1. Based on the AUC_{adj} value obtained with susceptible strains (0.09; SD ±0.02), we considered any sample with $AUC_{adj} > 0.13$ as positive to colistin resistance. In all cases, AUC_{adj} values increased with increasing ratio of resistant to susceptible organisms. Growth was detected at a maximum ratio of susceptible:resistant of 10^5:1, 10^4:1, 10^3:1, 10^2:1 and 10^1:1 for 43.7%, 12.5%, 18.5% and 12.5% and 12.5% of combinations, respectively. There was no difference in average AUC_{adj} between resistant strains with low (R1 and R2, colistin MIC= 4mg/L) and moderate (R3 and R4, colistin MIC= 8mg/L) levels of resistance (both AUC_{adj}= 0.39, Kruskal Wallis test, p= 0.688). The observed variation in AUC_{adj} values depended on both the choice of resistant and susceptible strains. In combinations with resistant strains, S2 yielded the lowest average AUC_{adj} (median 0.09 [1^{st} - 3^{rd} quartile, 0.07-0.29]) as well as the lowest limit of detection (average S:R

ratio of 10^2:1), whereas S4 gave the highest AUC_{adj} (median 0.62 [1st - 3rd quartile, 0.48-0.69]) as well as the highest limit of detection (average S:R ratio of 10^5:1).

Study flocks and their AMU

A total of 36 flocks (108 samples) were investigated in this study. The median flock size was 231 [1st - 3rd quartile, 189-401] chickens. Flocks were raised over a median of 19 [1st - 3rd quartile, 17-20] weeks. Colistin had been administered to 22/36 (61.1%) flocks. Among flocks given colistin, the average number of Animal Daily Doses (ADD) per 1,000 chicken-days of this antimicrobial administered over the production cycle was 149.5 Standard deviation [SD] ±261.6. Colistin was used more during the early flock cycle period (281.7 SD ±321.2 ADDs per 1,000 chicken-days) compared with the second period (17.4 SD ±18.1 ADDs per 1,000 chicken-days) (Wilcoxon paired test, $p<0.001$) (Table 1). This antimicrobial was administrated over a median of 4 [1st - 3rd quartile, 2-6] weeks. The data of colistin use among study flocks is displayed in Fig. S1.

In addition to colistin, a total of 27 non-colistin antimicrobials (belonging to 12 classes) were administered to study flocks. In decreasing order, oxytetracycline, tylosin, neomycin, ampicillin, streptomycin and doxycycline were the antimicrobials most used. The average number ADDs per 1,000 chicken-days of other antimicrobials among flocks using colistin was higher than flocks that did not use colistin (350.9 SD ±383.8 vs. 187.2 SD ±366.2, Wilcoxon test, $p= 0.004$). Among both type of flocks, antimicrobials were administrated more commonly during the first period (average No. ADD per 1,000 chicken-days 629.3 SD ±359.8 and 345.5 SD ±471.5, respectively) compared to the second period of chicken life (average No. ADDs per 1,000 chicken-days 72.5 SD± 98.5 and 29.0 SD ±48.6, respectively) (Table 1). Frequencies use of on-colistin antimicrobials in studied flocks were presented in Table S1.

Prevalence of colistin resistance at colony level

A total of 909 *E. coli* strains were isolated from 23 selected samples (~40 *E. coli* isolates/ sample) and were tested for their MIC against colistin. Among those, total of 129 strains (14.2%) were resistant to colistin. Of resistant strains, 75.2% strains had a MIC of 4 mg/L, whereas 24.0% had a MIC of 8mg/L. Only 1 isolate (0.8%) displayed a MIC of 16mg/L (Fig. S2). The beta-regression model that relates the AUC_{adj} (obtained from suspensions of 40 *E. coli* strains) to the percentage of resistant of those *E. coli* strains is shown in Fig. 2. The trend over AUC_{adj} was highly significant ($p< 0.001$). The equation $100/(1+e^{4.8-(7.04*AUCadj)})$ associated with this model was applied for estimating the prevalence of colistin resistance at colony level among field samples.

Changes of AUC_{adj} over production cycle and prevalence of colistin resistance

Overall, there was no significant change colistin resistance (AUC_{adj}) over the production cycle ($p= 0.569$, Fig. S3). Among flocks not exposed to colistin (n= 14), the differences AUC_{adj} between sampling points were small. However, among flocks using colistin (n= 22), the AUC_{adj} values for mid-production samples (0.54 [1^{st} - 3^{rd} quartile, 0.07-0.65]) were higher than those of day-olds (0.06 [1^{st} - 3^{rd} quartile, 0.04-0.52]) (Wilcoxon paired test, $p= 0.063$) and end of production samples (0.07 [1^{st} - 3^{rd} quartile, 0.06-0.55]) (Wilcoxon paired test, $p= 0.046$). There was little to no difference in AUC_{adj} values between day-old and end of production samples (Table 1).

The prevalence of colistin resistance at sample level was 38.9% [95%CI, 29.8-48.8%] (42/108 positive samples). The prevalence of resistance level of day-old, mid-, and end of production samples was 36.1%, 50.0% and 30.5%, respectively (χ^2 test, $p= 0.219$). The overall average estimated prevalence of resistance at colony level (generated from AUC_{adj}) was 19.4 SD ±26.3%.

147 Among flocks using colistin, the highest level of resistance corresponded to mid-production
148 samples (27.0 SD ± 26.4%), followed by day-old (15.7 SD ± 24.7%) and end production (12.8
149 SD ±18.1%) (Kruskal Wallis test, p= 0.070). In contrast, among non-using flocks, day-old
150 samples showed higher prevalence of resistance (28.8 SD ±36.0%) compared to mid (17.3 SD
151 ±28.7%) and end production (16.2 SD ±24.8%) (Kruskal Wallis test, p= 0.453). Summary results
152 are presented in Table 1 and individual sample results are given in Table S2.

153 **Risk factors for colistin resistance**

154 Table 2 shows results for univariable and multivariable analyses. In the multivariable model, use
155 of colistin during the two weeks prior to sampling (OR= 3.67; 95% [Confidence Interval] CI
156 0.68-19.7) and use of non-colistin antimicrobials (OR= 1.84; 95% CI 0.88-3.85) were associated
157 with colistin resistance at sample level.

158 **Estimation of test costs**

159 The reagent and media costs of broth microdilution and Etest for testing one sample based on the
160 investigation of 10 *E. coli* isolates were ~25 and ~63 US dollars (USD), respectively. The cost
161 for testing one sample by the growth-based method (based on 40 isolates) was ~6.5 USD. In
162 addition, broth microdilution involved a higher labour cost (average of ~1 person-day per
163 sample) compared with either the Etest or the growth-based method (~0.5 person-day) (Table
164 S3).

165 **DISCUSSION**

166 Here we developed a method that may be effectively used to quantify colistin resistance in
167 commensal *E. coli* in chicken flocks. Colistin is widely used in poultry and pig production
168 worldwide (4, 15, 16). In the Mekong Delta of Vietnam, colistin is typically administered to
169 chicken flocks in drinking water during the brooding period (1-4 weeks) with a prophylactic

purpose (i.e. to prevent disease) (5). Colistin is also included in some pig and poultry commercial feeds as a growth promoter (AGP) (17). However, from 2020 onwards, AGPs are longer be allowed in Vietnam (Law No. 32/2018/QH14), in line with legislative restrictions in Thailand (2015) (18), China (2016) (19) and India (2019) (20).

In contrast with the study of human patients, where colistin susceptibility testing is required to inform therapeutic choices (21) our method is aimed at estimating colistin resistance in mixed commensal *E. coli* populations. Through evaluation of the growth curves of standard *E. coli* suspensions from faecal samples, our method enables the detection of colistin resistance in a dichotomous fashion (presence/absence), as well as providing a quantitative assessment of colistin resistance at colony level (prevalence of resistant *E. coli*). The sensitivity of this methodology is, however, limited by the number of colonies harvested per sample (30-50), and may therefore miss colistin resistant strains in situations of very low prevalence. Indeed, statistically, given a sample of 40 colonies, there is a 5% probability of not detecting colistin resistance in any of them when the prevalence of resistant falls below 7.5%. Because of this, the method is more suitable advised for situations of medium to high prevalence of colistin resistance. The sensitivity could however be potentially increased by collecting several samples or increasing the number of *E. coli* colonies used in each suspension. For example, detection of a prevalence of 2% would require the investigation of 150 isolates (~4 samples, each with 30-50 colonies), detection of a prevalence of 1% would require 300 isolates (~8 samples); 0.1% a total of 3,000 isolates (~75 samples).

Although there was a statistically significant correlation between the prevalence of resistance and AUC_{adj}, we observed considerable variation in AUC_{adj} for similar prevalence values both in our laboratory validation as well as in our flock samples. This suggests variable growth capacity

among resistant strains, which may depend on their relative fitness. In the case of field suspensions containing a diversity of susceptible and resistant strains, it is also likely that the relative composition of strains may result in variable growth among the resistant strains due to the liberation of bacteriocin (i.e. colicins) in the culture media (22), or the presence of bacteriophages. This may also explain the variable limit of detection confirmed in laboratory conditions with different susceptible strains. In general, given identical prevalence of resistant strains, we observed higher AUC_{adj} values for individual susceptible-resistant strain combinations, compared to the specific mix of *E. coli* in field samples (Fig 2). It could be probably explained by less competition exerted in mixes containing a single strain, compared with heterogenous mixes containing ~40 different strains. Because of these reasons, prevalence estimates derived from AUC_{adj} should always be interpreted with caution.

We believe that our testing approach is more efficient than isolating and investigating individual colonies, at a relatively lower cost. However, it requires investment on a microplate reader costing between 3,000 and 10,000 USD. The technique presented here could potentially be adapted to the investigation of other types of phenotypic resistance in *E. coli* (i.e. tetracycline, ampicillin, etc.) but it would necessarily require optimizing working concentrations.

At the colony level, we obtained a median prevalence of 19.4% colistin resistance in flocks. These results are comparable with previous studies on chicken *E. coli* isolates in the area (12-22%) (7, 8). Furthermore, the observed ~40% resistance at sample level is consistent with a previous study on chickens in the Mekong Delta of Vietnam, where 5 *E. coli* colonies were investigated from each of 18 faecal samples (8). In such study, a total of 8/18 (44%) samples included at least one resistant strain (NT Nhung, personal communication). A PCR-based study

in this region reported that 59.4% chicken samples investigated tested positive for *mcr*-1 gene (7).

We demonstrated a short-term increase in phenotypic colistin resistance following administration of colistin use as well as non-colistin antimicrobials. This contrasts with a study conducted on a broiler flock in France, where administration of colistin failed to induce colistin resistance in Enterobacteriacea (including *E. coli*) (23). However, unlike in Vietnam, colistin use and resistance (including *mcr*-1) is relatively rare in European livestock (10). Overall, we found relatively high levels of colistin resistance (~40%), even in flocks that had not been given colistin (33.3%). There was evidence of colistin resistance in mid-production samples from flocks that had previously tested negative in day-old samples, and had not been administered colistin (3 of 8 flocks) (data not shown). This suggests that colistin resistance may have been generated or introduced to study flocks from other sources, such as contaminated water or feed, or due to contamination with bacteria from other animal species present in these small-scale farms.

Our findings of increased colistin resistance in flocks treated with antimicrobials other than colistin are intriguing. In a previous study on Mekong Delta pig farms, colistin resistance in *E. coli* strains was associated with use of non-colistin antimicrobials such as quinolones and cephalosporins (8). The presence of genes conferring for resistance against several different antimicrobial classes in *mcr*-harboring plasmids may explain these findings, and suggest that the use of non-colistin drugs may also select for colistin resistance (24).

We observed a peak of colistin resistance in mid-production samples among flocks using colistin, and generally levels of resistance decayed subsequently. This is likely to reflect the higher frequency of colistin use during the brooding period. A longitudinal study on travelers

238 colonized by *mcr-1*-carrying bacteria showed that they were able to completely eliminate these
239 bacteria within one month after returning to their home country (25). The reasons for a reduction
240 in resistance over time are unknown and may be due to a combination of factors leading to
241 plasmid loss and/or fitness costs. However, studies in the laboratory have shown that the
242 presence of plasmid-mediated colistin resistance has been shown to confer no fitness costs to *E.*
243 *coli* (26). It is worthwhile noting that in our study chicken flocks were of local native breed, and
244 they were typically raised over a 4-5 month period, a period much longer than that required by
245 industrial broilers (typically 1.5 months). This suggests that birds slaughtered earlier may have a
246 higher prevalence of colistin resistance, and this potentially represents an additional risk to the
247 consumer.

248 In summary, our method may be adapted to benchmark and monitor changes over time in colistin
249 resistance in faecal samples in other complex biological systems such as abattoirs, slaughter-
250 points and sewage, or even in human individuals. Our results indicate a high background of
251 colistin resistance even in flocks not using this antimicrobial. The observed increases after
252 colistin use were short-lived and suggest that in small-scale farming systems reducing colistin
253 resistance may require increasing biosecurity as well as restocking colistin-negative day-old
254 chicks.

255 **MATERIALS AND METHODS**

256 **Study design**

257 In order to investigate the biological basis and the limit of detection of the proposed method,
258 we used four previously characterized *mcr*-1 colistin resistant *E. coli* strains, two displaying
259 moderate-level (MIC= 8mg/L) and two low-level (MIC= 4mg/L) colistin resistance, alongside
260 four colistin-susceptible strains. We prepared standard bacterial suspensions consisting of a

mix of each of the resistant and the susceptible strains at different ratios; these were incubated in medium with and without 2mg/L of colistin. A growth curve from each suspension was obtained by measuring the optical density (OD_{600nm}) during incubation. The area under the curve (AUC_{adj}) of each colistin-containing standard suspension was adjusted by the AUC values obtained from its equivalent colistin-free suspension. We investigated the relationship between the prevalence of resistance at colony level and the observed AUC_{adj} from the examination of 30-50 individual *E.coli* isolates from each of 23 samples and obtained a model equation. We calculated AUC_{adj} values of suspensions consisting 30-50 *E. coli* colonies harvested from each of 108 pooled faecal samples from 36 small-scale (single-age) chicken flocks raised in Dong Thap province (Mekong Delta, Vietnam) (27). We inferred the prevalence of resistant *E. coli* in flock samples investigated by extrapolation using the model equation. The contribution of colistin use and other antimicrobials administered to flocks on the observed phenotypic colistin resistance was investigated by building logistic regression models with age as primary time variable.

Culture of standard suspensions; calculation of the AUC_{adj} and limit of detection

Each of the chosen resistant *E. coli* strains (named R1 to R4, where R1 and R2 had MIC= 4mg/L; R3 and R4 had MIC= 8mg/L) and susceptible (all MIC≤ 1mg/L) strains (S1 to S4) were incubated in cation adjusted Mueller Hinton II Broth II (MHB2, Sigma-Aldrich, USA) at 37^{o}C, 200 rpm for 4h (log-phase) and these bacterial inoculum were adjusted to 10^8 CFU/mL (OD_{600nm}= 0.1), and then diluted down with MHB2 to 10^6 CFU/mL. Each susceptible strain was mixed with a resistant strain, giving a total of 16 combinations with susceptible: resistant ratios ranging from 1:0 (susceptible strain only) to 0:1 (resistant strain only). Intermediate ratios were 10^1:1, 10^2:1, 10^3:1, 10^4:1, and 10^5:1. A total of 100μL of each suspension was

added into a well of polystyrene microplate (Corning, USA), containing 100μL of colistin solution (final working concentration was 2mg/L). In addition, respective colistin-free (control) suspensions were prepared. Plates were incubated in a microplate reader (SPECTROStar, BMG Labtech, Germany) at 37^{o}C for 20h, and the turbidity (OD_{600nm}) readings were recorded every hour. All experiments were conducted in triplicate.

The areas under the curves (AUC) generated over the 20-hour observation period were computed. The AUC value generated from each standard suspension ($AUC_{[i]}$) was related to the AUC generated by its respective colistin-free control (AUC_{adj}= $AUC_{[i]}$/$AUC_{[0]}$). Samples with AUC_{adj} greater than the average value obtained with each of the four susceptible strains +2 SD were considered positive to colistin resistance.

Flock sample and AMU data collection

Fresh pooled faecal samples were collected from each flock at three time-points: (1) day-old chicks, (2) mid-production (~2-3 months-old) and (3) end of production (~4-6 months-old). Day-old faecal (i.e. meconium) samples were collected from the crates at the time when chicks were delivered to the farms. For mid- and end-production sampling, sterile paper liners were placed near drinkers and feeders in the chicken house/pen to collect deposited droppings. After a minimum of 10 droppings had been deposited, liners were swabbed using sterile gauzes. Each of collected gauze was placed in a universal jar and mixed vigorously with 50mL saline buffer. One ml of the resulting eluate was stored at -20^{o}C with glycerol. Data on AMU had been collected using purposefully designed diaries where farmers were asked to note down all antimicrobials used. Farmers were instructed to keep all packages of antimicrobials used on their flocks (5). Sample and data collection were conducted between October 2016 and October 2018.

Testing of pooled faecal samples

Eluates from pooled faecal samples were plated onto ECC agar (CHROMagar, France) and incubated at 37°C for 20h. A total of 30-50 *E. coli* (blue) colonies from each agar sample were picked, pooled and incubated in CAMHB to log-phase. The resulting bacterial suspensions were investigated as described above.

Estimation of the prevalence of colistin resistance at colony level

We selected a number of positive samples with variable levels of AUC_{adj}. From each sample, 40 *E. coli* were isolated and tested individually for colistin MIC by standard broth micro-dilution. These pools of 40 *E. coli* were also investigated for their AUC_{adj} as described previously.

Data analyses and cost estimation

In order to relate the AUC_{adj} value to the measured prevalence of resistance among selected samples, we fitted a beta-regression model using the '*betareg*' package in R (28). Both the trend and the dispersion were allowed to vary over AUC_{adj} in a linear way.

AMU in flocks was quantified for the two periods defined by the sampling schedule: (1) between restocking and mid-production, and (2) between mid- and end of production. Weekly estimates of colistin use were expressed as the number of ADDs (number of Animal Daily Doses administered per 1,000 chicken days) calculated for each of the two periods (5). Risk factors associated with colistin resistance at mid- and end of production were investigated by logistic regression. The outcome was colistin resistance (Yes/No) at sample level. The variables investigated were: (1) Age of chicken flock (weeks); (2) Use of colistin within two weeks prior to sampling (Yes/No); (3) Number of ADDs per 1,000 chicken-days of colistin in each period; (4) Colistin resistance of day-old chicks (Yes/No); and (5) Number of ADDs per 1,000 chicken-days of non- colistin antimicrobials used in each period. The variable Age of chicken flock was

included in all univariable models because it is the principal time variable. Since we had two measurements per flock (mid and end cycle samples), we used generalized estimation equations with an exchangeable correlation structure to estimate the parameters using the '*geepack*' R package (29, 30).

The change in AUC_{adj} over age of chicken was modeled using a random effects linear regression. In order to allow for a nonlinear trend, we used a natural spline for the fixed effect term (knots at 0, 8, 12 and 20 weeks). We allowed for a random intercept and linear trend by age.

The overall costs (per sample) of the method described above were calculated based on expenses on medium, reagents and consumables (excluding staff time, which was estimated separately). The estimated costs were compared with those incurred in testing one sample by broth microdilution and Etest in Vietnam as of January 2020. Our calculations were based on the investigation of 40 *E. coli* isolates per sample using the growth-based method, compared with 10 isolates each by broth microdilution and by Etest.

SUPPLEMENTAL MATERIAL

Table S1 Non-colistin antimicrobials used in studied flocks

Table S2 Estimated percentage of resistant *E. coli* from 108 samples

Table S3 Estimated costs (in US dollar) of testing 1 sample to determine colistin phenotypic resistance of *E. coli*

FIG S1 Usage of colistin among study flocks by week.

FIG S2 Distribution of MIC values among 909 *E. coli* isolates

FIG S3. Changes in AUC_{adj} over time (weeks) in chicken flocks

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357 We declare that we have no competing interests.

358 N.T.N and JC-M conceived the idea; J.C, G.T and S.B advised on the study design; N.V.C and
359 B.T.K coordinated field sampling and data collection; N.T.N, N.T.P.Y. and N.V.K.T performed
360 laboratory experiments. N.T.N, R.B.G and J.C-M conducted data analyses and produced first
361 draft. All authors commented on subsequent versions.

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456 TABLE 1 Description of AMU and estimated prevalence of colistin resistance in 36 small-scale

457 chicken flocks stratified by whether farmers administered colistin or not.

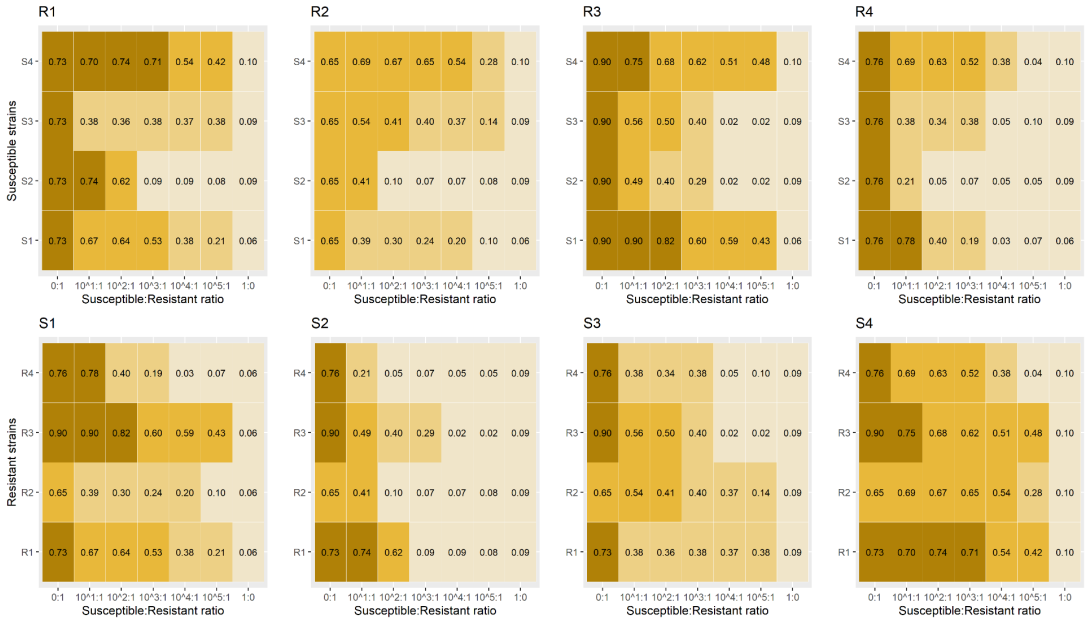
	Flocks not using colistin (n=14)	Flocks using colistin (n=22)	All flocks (n=36)
Cycle duration (weeks) (median [1st - 3rd quartile])	19 [17-20]	20 [17-21]	19 [17-20]
No. chickens (median [1st - 3rd quartile])	249 [194-482]	208 [128-398]	231 [189-401]
No. ADDs of colistin (per 1,000 chicken-days) (mean ± SD)			
First period	0	281.7 ±321.2	172.1 ± 285.1
Second period	0	17.4 ±18.1	10.6 ± 16.4
Whole production cycle	0	149.5 ±261.6	91.4 ± 216.4
No. ADDs of non-colistin antimicrobials (per 1,000 chicken-days) (mean ± SD)			
First period	345.5 ± 471.5	629.3 ±359.8	518.9 ± 424.2
Second period	29.0 ± 48.6	72.5 ± 98.5	55.6 ± 84.7
Whole production cycle	187.2 ±366.2	350.9 ± 383.8	287.3 ± 382.9
No. flocks using colistin two weeks prior to			
Mid-sampling	0	11	11
End of sampling	0	1	1
AUC_{adj} (median, [1st - 3rd quartile])			
Day-olds	0.07 [0.04-0.42]	0.06 [0.04-0.52]	0.07 [0.04-0.65]
Mid-production	0.06 [0.03-0.43]	0.54 [0.07-0.65]	0.20 [0.05-0.63]
End of production	0.07 [0.06-0.55]	0.07[0.06-0.55]	0.07 [0.05-0.56]
Prevalence of resistance (%) at sample level (95% CI)			
Day-olds	42.8 (18.8-70.3)	31.8 (14.7-54.9)	36.1 (21.3-53.8)
Mid-production	28.6 (9.5-58.0)	63.6 (40.8-82.0)	50.0 (34.5- 65.5)
End of production	28.6 (9.5-58.0)	31.8 (14.7-54.9)	30.5 (16.9- 48.3)
Estimated prevalence of resistance (%) at colony level (mean ± SD)			
Day-olds	28.8 ±36.0	15.7 ±24.7	20.8 ±29.8
Mid-production	17.3 ±28.7	27.0 ±26.4	23.3 ±27.4
End of production	16.2 ±24.8	12.8 ±18.1	14.1 ±20.7

458 AUC= area under the growth curve; CI= Confidence interval; SD= standard deviation

459 TABLE 2 Logistic regression models investigating risk factors associated with colistin resistance
460 in chicken flocks at sample level. Models were based on a total of 72 samples (mid and end
461 production); 29 were positive resistance to colistin.

Variable	Univariable[a]			Multivariable		
	OR	95% CI	p-value	OR	95% CI	p-value
Age of chicken flock (weeks)	0.93	0.84-1.02	0.156	1.04	0.91-1.18	0.605
Use of colistin within last two weeks (Yes/No)	5.30	1.17-24.08	0.030	3.67	0.68-19.70	0.128
No. ADDs per 1,000 chicken-day of colistin[b]	1.66	1.00-2.76	0.049	1.06	0.55-2.06	0.845
Colistin resistance of day-old chicks (Yes/No)	1.45	0.53-3.97	0.461	1.61	0.54-4.84	0.395
No. ADDs per 1,000 chicken-day of non-colistin antimicrobials[b]	2.10	1.18- 3.73	0.012	1.84	0.88-3.85	0.102

462 [a]The variable 'Age of chicken flock' was included in all univariable models to calculate estimates for all subsequent
463 variables. [b]logarith transformed after adding 1, ADD= animal daily dose; OR= Odds ratio; CI= Confidence interval.



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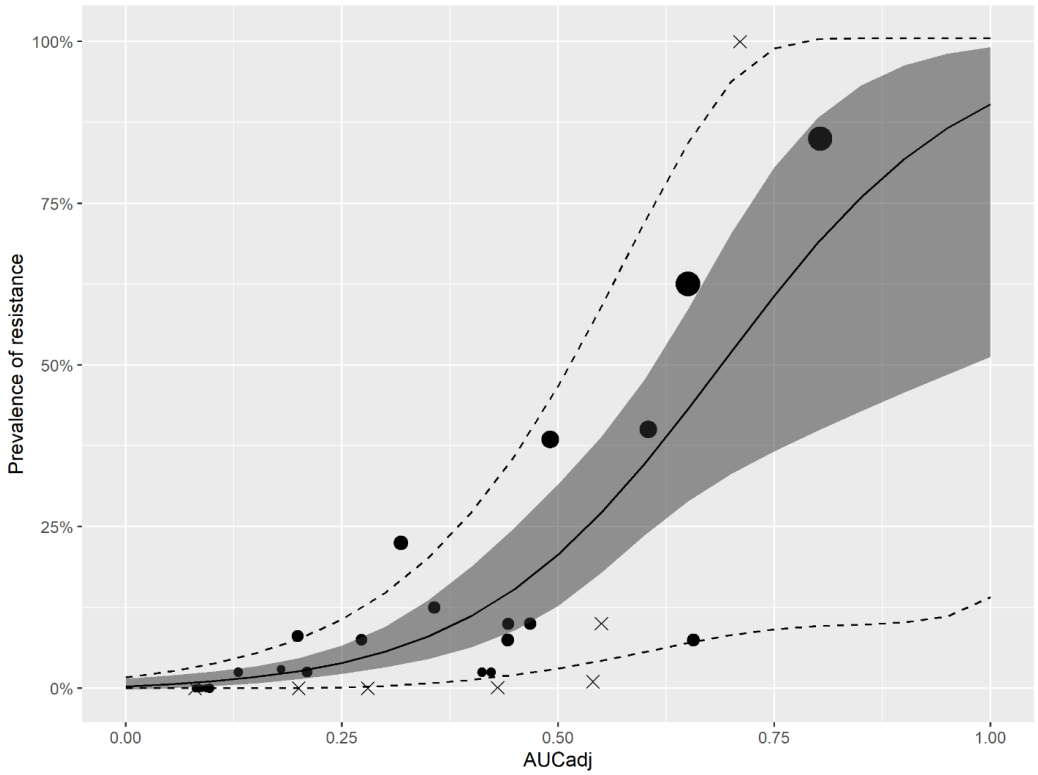
465 FIG 1 AUC_{adj} of standard suspensions. Positive growth values are represented by increasing strength of color. R= Resistant, S=

466 Susceptible. Average of AUC_{adj} of resistant strain 1, 2, 3 and 4 was 0.40, 0.30, 0.41 and 0.26, respectively. Average AUC_{adj} of

467 susceptible strain 1, 2, 3 and 4 was 0.41, 0.19, 0.31 and 0.54 respectively.



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469 FIG 2 Relationship between AUC_{adj} (from mix of 40 *E. coli* per sample) and prevalence of
470 colistin resistance at colony level. The figure shows the predicted mean value of resistance with
471 pointwise 95% confidence as shaded area. The dotted lines give the 5% and 95% prediction
472 intervals. Circle symbols indicated AUC_{adj} values of mixed *E. coli* in field samples. Size of dot
473 represented the average MIC of each sample. Cross symbols indicated AUC_{adj} values of mixed
474 susceptible and resistant strains.

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Reducing Antimicrobial Usage in Small-Scale Chicken Farms in Vietnam: A 3-Year Intervention Study

Doan Hoang Phu[1,2], Nguyen Van Cuong[1], Dinh Bao Truong[1,2], Bach Tuan Kiet[3], Vo Be Hien[3], Ho Thi Viet Thu[4], Lam Kim Yen[5], Nguyen Thi Tuyet Minh[6], Pawin Padungtod[6], Erry Setyawan[7], Guy Thwaites[1,8], Jonathan Rushton[9] and Juan Carrique-Mas[1,8]*

[1] Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2] Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam, [3] Sub-Department of Animal Health and Production, Cao Lãnh City, Vietnam, [4] Department of Veterinary Medicine, Can Tho University, Can Tho, Vietnam, [5] Faculty of Agriculture and Aquaculture, Dong Thap Community College, Cao Lãnh City, Vietnam, [6] Food and Agriculture Organization of the United Nations, Hanoi, Vietnam, [7] Food and Agriculture Organization of the United Nations, Jakarta, Indonesia, [8] Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom, [9] Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom

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Ioannis Magouras, City University of Hong Kong, Hong Kong

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***Correspondence:**
Doan Hoang Phu
phudh@oucru.org

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Indiscriminate antimicrobial use (AMU) in animal production is a driver of antimicrobial resistance globally. There is a need to define sustainable interventions to reduce AMU in small-scale production systems, which currently represent the most widespread farming systems in South East Asia and many low- and middle-income countries. We conducted a before-and-after intervention study on a random sample of small-scale chicken farms in the Mekong Delta of Vietnam from 2016 to 2019. The study included a baseline followed by an intervention phase where farmers were provided with regular veterinary advice on flock health and husbandry, as well as antimicrobial replacement products. Of 102 recruited farms (raising >100 chickens per flock cycle), thirty-five (34.2%) entered the intervention phase, whilst the rest stopped raising chickens, mainly due to suboptimal flock performance. Through the implementation of our intervention, chicken flocks reduced levels of AMU by 66% [adjusted hazard ratio (HR) = 0.34; $p = 0.002$) from a baseline of 343.4 Animal Daily Doses per 1,000 chicken-days and decreased weekly mortality by 40% (adjusted HR = 0.60; $p = 0.005$) from a baseline mortality of 1.60 per 100 birds. Chicken bodyweight increased by 100 g ($p = 0.002$) in intervention flocks. Our findings demonstrate that the provision of veterinary advice can achieve substantial reductions in AMU in small-scale production systems without compromising flock health and productivity.

Keywords: antimicrobial use, disease, smallholder farms, poultry, Vietnam

INTRODUCTION

In many low- and middle-income countries (LMICs) small-scale poultry farming plays a crucial role in supporting the livelihoods of rural communities (1). Compared with other species, poultry production has relatively low investment and production costs (2). Globally, poultry (mainly chicken) is the second most consumed type of meat (117 million tons in 2017), and by 2026 it is expected to surpass pork (3).

Antimicrobial use (AMU) in animal production has been recognized as a driver of antimicrobial resistance (AMR) globally (4, 5). In terms of frequency, chickens are the target of the highest AMU levels of all animal food species (6). In addition, many antimicrobial active ingredients (AAI) regarded as critically important for human medicine by the World Health Organization (7) are often used in chicken production (8).

In Vietnam, it has been estimated that three quarters (72%) of all AMU (3,842 tons in 2015) are aimed at animal production (9). Studies in the Mekong Delta region of Vietnam have described very high amounts of antimicrobial to small-scale chicken flocks (8, 10–12). The high levels of disease in flocks in the area is a major driver of AMU in such systems (13). In chicken farms, antimicrobials are used primarily for disease prevention (10), since farmers regard them as a cheaper alternative to other disease control measures (14). Recent studies have shown that some of the most commonly used AAIs in small-scale chicken flocks in the area also belong to the WHO highest priority, critically important antimicrobial classes such as polymyxins and fluoroquinolones (8, 12, 15). This situation is aggravated by a general lack of awareness about antimicrobials and the negative consequences of AMR among farmers (16). In addition, the ease of access to antimicrobials over-the-counter in veterinary drug shops (17) and their affordability (18) are factors that contribute to excessive AMU in Vietnam.

There is a pressing need to identify sustainable interventions that reduce AMU in food animal production systems. Such interventions will need to overcome the diversity of production systems and value chains they depend on and the patterns of AMU in these systems and their policy contexts. A number of interventions have already taken place in developed countries based on improvements in biosecurity and husbandry practices aiming at reducing AMU in pigs (19–21) and broilers (22). However, no intervention studies targetting AMU in small-scale farming systems from LMICs have been published. We conducted a "before-and-after" randomized intervention study on small-scale chicken farms in the Mekong Delta region of Vietnam. The intervention consisted of providing farmers with regular veterinary advice, alongside antimicrobial replacement products (23). The aim was to investigate the impact of this intervention on AMU, as well as on flock disease and productivity. Results and the lessons from this study can be adapted to comparable animal production systems in Vietnam and more generally, to other LMICs.

MATERIALS AND METHODS

Study Design

The intervention was designed as a randomized "before-and-after" controlled study on small-scale farms raising chickens for meat in two districts (Cao Lanh and Thap Muoi) within Dong Thap province (Mekong Delta, Vietnam) (**Figure 1** and **Supplementary Figure 1**). We aimed to recruit farmers raising chicken flocks (defined as a group of birds raised together in the same building) meeting the criterion ">100 meat chickens raised as single age."

Our small-scale commercial flocks lie between "backyard" flocks and intensively managed "industrial" systems, roughly corresponding to FAO Sectors 2 and 3 (between 50 and 2,000 birds, with feed and water supplied to the birds) (24). The study was designed in two stages, a "baseline" followed by an "intervention" phase. Two intervention arms (Arm 1 and Arm 2) were initially planned, both including the provision of training and advice to farmers, as well as a control arm (Arm 3) (no training or advice). The difference between both intervention arms was that Arm 2 also included the withdrawal of medicated commercial feed. This aimed at investigating whether restriction of medicated feed might have affected disease outcomes, therefore contributing to changes in levels of AMU (23).

Farmers registered in the official SDAH census (2014) were contacted by post and were invited to participate in an introductory meeting held in October 2016 in each of the two study districts. In these meetings, the project aims and methods were outlined. Farmers willing to enroll in the study were asked to contact project staff as soon as they restocked with day-old chicks.

Description of the Baseline and the Intervention

During the baseline phase of the study, routine AMU and productivity data were collected from enrolled farms without the provision of any advice. Using a random number generator, we allocated enrolled farms to either an intervention or a control arm. All farms allocated to the intervention arm were supported with a Farmer Training Programme (FTP), where farm owners were invited to participate in six workshops where a poultry veterinarian instructed them on the principles of chicken husbandry, prevention, control of infectious diseases and waste management and a Farm Health Plan (FHP), where each farm was assigned to a Project Veterinarian (PV) who was responsible for providing specific advice to farmers. The PV visited each farm on three different occasions for each flock cycle: (i) early-brooding (weeks 1–2), (ii) late brooding (weeks 3–4), and (iii) grow-out (>2 months) periods. Prior to each visit, the PV reviewed records of productivity and disease over previous cycles, inspected the flock and house/pen, reviewed farmers' records, discussed with farm owner about current production/health issues, and then drafted a list of recommendations to address them. In addition, the PV recommended the farm owner to use an antimicrobial replacement product, either a liquid phytogenic solution containing essential oils (Product A) or a yeast fraction-containing product (Product B) for 3 days a week over the first 10 weeks of the production cycle. Product A was recommended to flocks with a history of diarrhoeal disease in previous cycles; for all other flocks the PV recommended Product B. By providing these products we aimed at allaying the farmers' anxiety about reducing or eliminating antimicrobials during the early phase of production, which is critical in terms of disease and mortality. In all visits, the PVs reminded the farmers that healthy birds should not be given any antimicrobials.

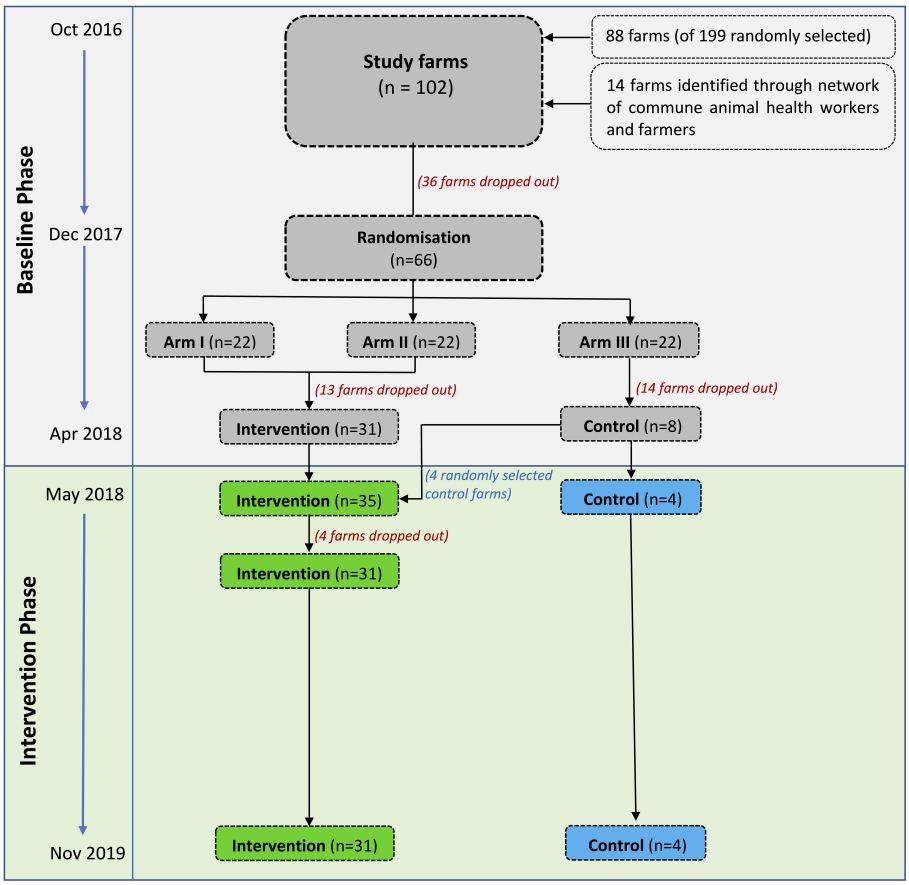

FIGURE 1 | Recruitment and follow-up of study farms. Modified from design of project named ViParc published in 2017 (23).

Data Collection

Each farmer was provided by project staff with a diary to weekly record data on farming practices, including number of chickens purchased, number of chickens in and out of the flock (number of dead and sold chickens), as well as the types and quantities of antimicrobial products used. The average bodyweight of slaughter-age chickens was also measured by average of total bodyweight of chickens divided for total number of chickens sold. Project staff visited study farms four times (different from PV visits) to verify the data collected, which was subsequently transferred to validated questionnaires and double-entered into a web-based database.

Statistical Analyses

The initially proposed sample size was based on previous quantitative data on AMU in Mekong Delta chicken farms (10). We aimed at recruiting 120 farms and estimated a total of 40 farms for each arm. A sample size of 40 farms per arm, each contributing with 2 cycles investigated during baseline and 2 during the intervention, and a two-sided significance level of 5%, will have 82% power to detect a ∼33% reduction, and a 91% power to detect a 50% reduction. Since the study design exploited within-farm correlation of unknown magnitude, the true power was expected to be higher.

The primary outcome was the weekly number doses of antimicrobial active ingredient (AAI) corresponding to 1 kg of live chicken administered to a flock (Weekly ADD_{kg}). Secondary outcomes were "Weekly mortality," calculated by dividing the number of chickens dying over the week by the total chicken present at the beginning of each week (%), and "Weight of the birds (in units of 100 g) at the time of sale." The latter was calculated by dividing the total flock weight by the number of chickens sold at the end of the cycle. The correlation between all three outcomes at flock and at week level was investigated using the Spearman's rank correlation coefficient.

Weekly antimicrobial consumption in each flock was expressed as No. of Animal Daily Doses-kilogram (ADD_{kg}). ADD_{kg} was calculated for each antimicrobial contained in each product based on the preparation instructions included in its technical dossier/label. The amounts of antimicrobial product administered each week were multiplied by a dilution factor [for water-administered products, Volume of water (ml)/Weight of product (g); for feed feed administered products, Weight of feed (g)/Weight of product (g)]. The obtained amounts were

then divided by the estimated weekly water of feed consumption corresponding to a 1 kg chicken (7*0.225 l of water, 7*0.063 kg of feed). The number of ADD_{kg} per 1,000 kg chicken-days on any given week was calculated by dividing the total of number of ADD_{kg} by the estimated weight of the flock at a given week, and then multiplied by 1,000. The estimated total weight of the flock by week was calculated by multiplying the total number of chickens at the beginning of the week by their estimated weight. The No. ADD_{kg} per 1,000 chicken-days can be interpreted as the number of days (per 1,000) when one chicken is treated or the number of treated chickens daily (per 1,000) (8).

$$\text{No.ADD}_{kg} = \frac{\text{Amount of antimicrobial product administered } (g) * \text{dilution factor in water (l/g) or feed (kg/g)}}{\text{Daily consumption of water (0.225l) or feed (0.063kg) of 1 kg chicken}}$$

$$\text{No.ADD}_{kg}\text{per 1,000 kg chicken} - \text{days} = \frac{\text{No. ADD}_{kg}}{\text{Estimated weight (kg) of the flock}} * 1,000$$

We built Poisson regression models with for "Weekly ADD_{kg}" and "Weekly mortality." For the former the offset was the (weekly) total number of chicken-kg days (log); for the latter it was the number of chickens at the beginning of the week (log). In addition, a linear regression model was developed with bodyweight of chickens at the point of sale (kg) as outcome. In all cases, "Farm," "Flock cycle" and "Week" were modeled as random effects, where "Week" was nested within "Flock cycle," and the latter was nested within "Farm." The main variable of interest was the impact of the intervention delivered; therefore, we investigated "Status" (baseline, transition, and intervention) as an explanatory variable in Intervention Arm and "Status" (baseline, intervention calendar time) as an explanatory variable in the Control Arm. "Status = transition" was assigned to those flocks that were not exposed to all three advisory visits for Intervention Arm farms. This occurred to a number of flocks at the beginning of the intervention phase, given that some advisory visits (typically the first and second) were missed. In order to account for the potential confounding effects of "District" and "Flock size" these were forced into a multivariable model; we tested the interactions between "Status = intervention" with "District" and "Flock size" to investigate whether the observed effects were dependent on the geographical location or the size of the flock. Moreover, we investigated whether subsequent cycles over the intervention resulted in improved outcomes by splitting "Status = intervention" into "Status = first intervention cycle" and "Status = subsequent intervention cycle." The presence of overly influential observations was investigated by testing the model with and without those observations yielding the largest residuals. We used the "survey" package to calculate (farm-flock-week) adjusted estimates and "lme4" package to build statistical models (http://www.r-project.org).

RESULTS

Recruitment of Study Farms

The study took place between October 2016 and November 2019. A meeting with 199 randomly selected farmers from the farm census registered as owners of chickens was held in October 2016. Eighty-eight participating farmers indicating their willingness to restock within 6 months were enrolled. The remaining 14 farms were identified by commune animal health workers or through contact with farmers that had already been enrolled in the study. Therefore, a total of 102 farms were enrolled over the period October 2016 to October 2017. The baseline phase spanned October 2016 to April 2018. The intervention was delivered from May 2018 to November 2019.

The flow of participating farms was complicated by many ($n = 63$) that stopped farming during the study for financial reasons unrelated to the study. The recruitment and allocation to arms is summarized in **Figure 1**. Their location is presented in **Supplementary Figure 1**.

In December 2017 farms that remained in production at the time ($n = 66$) were randomized to either intervention Arm 1 ($n = 22$), intervention Arm 2 ($n = 22$) or a Control arm ($n = 22$). Following discussion with the farmers, it became apparent that replacement of medicated feed as initially planned for Arm 2 would not be acceptable; therefore, the two intervention arms were merged into one single arm. At the time of the onset of the intervention (May 2018), of 44 farms initially allocated to the intervention, only 31 remained in business; of the 22 allocated to the control, only 8 were still raising chickens. To compensate for the reduced sample size and associated loss in study power, we further allocated four randomly-selected control farms to the intervention arm. Therefore, a total of 35 and 4 farms allocated to the intervention and control arm, respectively, proceeded to the intervention phase (**Figure 1**).

The intervention commenced with the delivery of the Farmer Training Programme (FTP) in May 2018 to owners of the 35 intervention farms; however, at that time 18 had already restocked with day-olds. Since flocks ($n = 22$) in these farms were not exposed to all four advisory visits, they were therefore analyzed as "transition" flocks. Four farms assigned to the intervention arm stopped raising chickens shortly after having attended the FTP modules, and were classified as "Baseline-Transition-Stop" farms.

Data collected from 35 farms (31 intervention, 4 control) were eligible for the final analyses. One hundred flock cycles were analyzed as baseline phase (87 in intervention; 13 in control arms) and 89 flock cycles corresponded to the intervention phase (77 intervention farms; 12 in control farms). Of the 77 flocks, 28 (14 farms) were given Product A (an essential oil); and 43 (14 farms) were given Product B (a yeast fraction-based product). Six flocks (3 farms) did not agree with the supplementation of either Product A or Product B.

The median number of chickens restocked per flock was 303 [IQR (inter-quartile range) 200–500], and the median duration

TABLE 1 | Categorization of farms based on number of flocks investigated during the baseline phase, transition period, and intervention phase.

Farm group	No. farms (%)	No. flock cycles by status(%)				
		Baseline	Transition	Intervention	Control	Total
Baseline-Transition-Stop	4 (3.9%)	9	4	–	–	13 (3.9%)
Baseline-Transition-Intervention*	14 (13.7%)	42	18	37	–	97 (29.4%)
Baseline-Intervention*	17 (16.7%)	45	–	40	–	85 (25.8%)
Baseline-Control*	4 (3.9%)	13	–	–	12	25 (7.6%)
Baseline-Stop	63 (61.8%)	110	–	–	–	110 (33.3%)
Total	102 (100%)	219	22	77	12	330 (100%)

**Data used in further statistical modeling.*

of one production cycle was 18 weeks (IQR 16.0–20.0). Each farm raised a median of 5 flocks (IQR 4.0–7.0), 2 (IQR 1.0–2.2) during the baseline and 2 (IQR 1.0–2.5) during the intervention phase. Details of number of flocks per farm and status are shown in **Table 1** and **Figure 2**. Descriptive characteristics of chicken farms by total farms, flocks and weeks were presented in **Supplementary Table 1**.

AMU, Mortality, and Bodyweight of Chicken Flocks

We collected data over 5,872 production-weeks for all study flocks combined; of which 3,899 (66.4%) corresponded to the baseline and 1,973 (33.6%) to the intervention phase. The latter included 396 (6.7%) weeks from transition flocks, 1,350 (23.0%) weeks of full intervention flocks, and 277 (3.9%) weeks from flocks allocated to the control arm. Data on AMU, mortality and bodyweight in these flocks over the baseline, transition and intervention cycles are presented in **Table 2**.

During the baseline phase, flocks ($n = 110$) raised in the 63 farms that dropped out prior to the implementation of the intervention phase had a higher mortality (weekly average 3.18 per 100 birds; SE ± 0.3), than flocks ($n = 109$) in 39 farms that proceeded to the intervention (1.52 per 100 birds; SE ± 0.1) (Wilcoxon Test, $p = 0.020$).

The weekly summary data of the outcome variables and the distribution of flocks concerning these in flocks during the baseline ($n = 87$) and intervention phases ($n = 77$) are displayed in **Figure 3**. Weekly AMU in these flocks was reduced from 343.4 (SE ± 33.5) (baseline) to 223.9 (SE ± 30.0) (intervention) Animal Daily Doses (ADD_{kg}) per 1,000 kg chicken-days (−34.8%) (one-sided Wilcoxon test, $p < 0.001$). The bodyweight at slaughter-age of chickens of intervention flocks was 1,670 g (SE ± 30), compared with 1,560 g (SE ± 20) during baseline (+7.1%) (one-sided Wilcoxon test, $p = 0.006$). However, weekly mortality increased from 1.60 (per 100 birds) (SE ± 0.2) to 1.64 (SE ± 0.2) (+2.4%), although the difference was not significant (one-sided Wilcoxon test, $p = 0.999$).

The unadjusted overall mortality increased slightly during the intervention. However, the number of farms that experienced a reduction in mortality exceeded (19/31) than those that increasing it (12/31). The changes in (flock average) values of ADD_{kg} per 1,000 kg chicken-days, mortality and bodyweight between the baseline and intervention phases are displayed in **Figure 4**. Among intervention flocks, there were 3/77 (3.9%) with an average weekly mortality greater than 12% (12.8, 24.8 and to 26.0%) and a cumulative mortality of >98%; two of these flocks were detected with Highly Pathogenic Avian Influenza (HPAI) and one with *Avibacterium paragallinarum,* compared with 2/87 flocks experiencing >10% weekly mortality among baseline flocks (one 12.4% and one 12.8%) and cumulative mortality of 100% in these two baseline flocks.

In the four farms that were allocated to the control arm, a total of 13 flocks were investigated during the baseline phase, and 12 during the intervention phase. AMU in these decreased from 216.8 (SE ± 71.8) to 182.5 (SE ± 56.3) (Wilcoxon Test, $p = 0.857$); weekly mortality changed from 1.17 to 1.29 per 100 birds (Wilcoxon test, $p = 0.493$) and bodyweight changed from 1,680 to 1,600 g (Wilcoxon test, $p = 0.511$).

Correlation Between AMU and Mortality, Bodyweight

There were significant correlations between weekly AMU (ADD_{kg} per 1,000 kg chicken-days) and mortality (Spearman's rank correlation $R = 0.26$; $p < 0.001$). There were, however, no correlations between average bodyweight and AMU ($R = -0.06$, $p = 0.292$) or mortality ($R = -0.09$, $p = 0.128$) at flock level. The details of these calculations are provided in **Supplementary Figure 2**.

Modeling

The statistical models investigating the effectiveness of the intervention on AMU, mortality and chicken bodyweight are presented in **Table 3**. In the univariable models for the Intervention Arm, "Status = intervention" was associated with an overall decreased AMU (HR = 0.33; 95% CI = 0.17–0.65; $p = 0.001$) (−67%), decreased mortality (HR = 0.57; 95% CI = 0.40–0.82; $p = 0.002$) (−43%) and increased bodyweight (+100 g; 95% CI 37–164 $p = 0.002$). The size of the flock was negatively associated with AMU (HR = 0.55, 95% CI 0.36–0.85, $p = 0.007$), but positively associated with mortality (HR = 1.34; 95% CI 1.02–1.47; $p = 0.032$). Adjustment for flock size resulted in minimal change in the estimates of AMU (−66%) (HR = 0.34; 95% CI 0.18–0.66; $p = 0.002$), mortality (−40%)

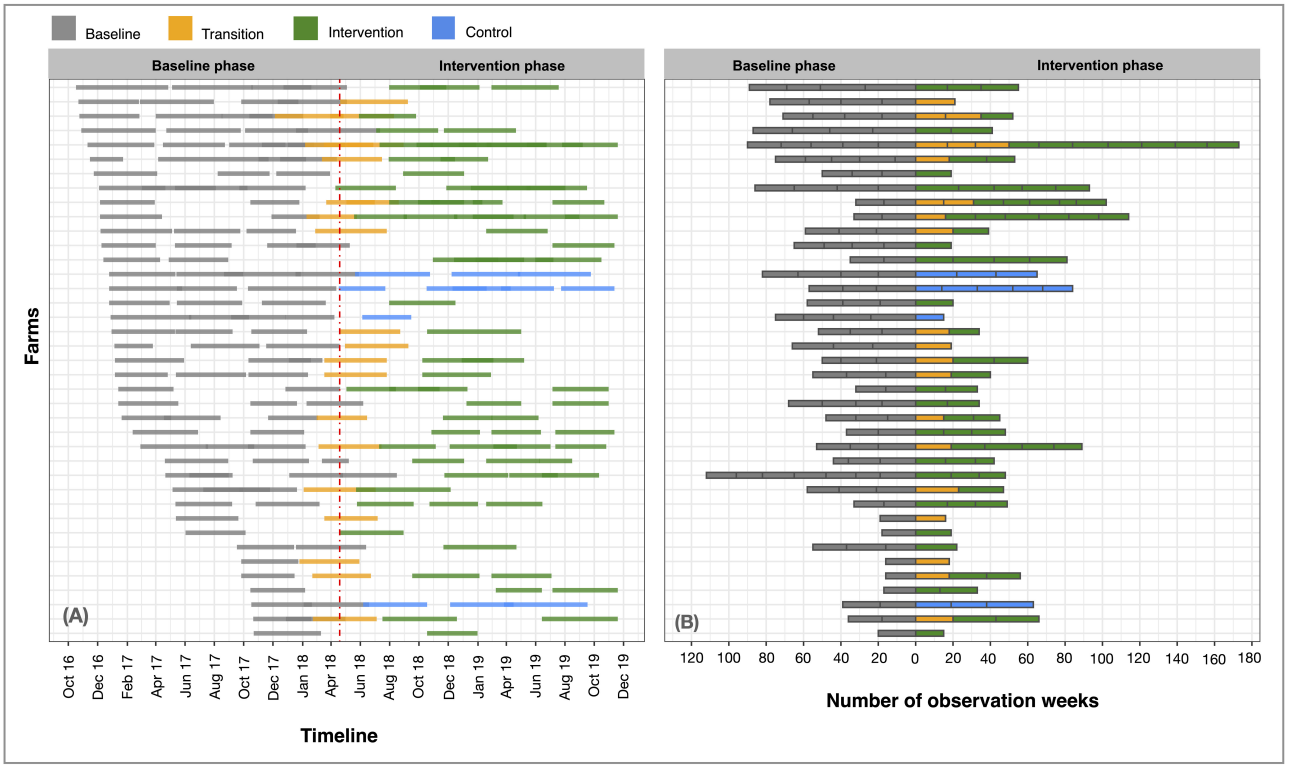

FIGURE 2 | Calendar and observation time of the 39 farms recruited during the baseline and intervention phases. These included: Baseline-Transition-Stop farms (n = 4), Baseline-Control farm (n = 4), Baseline-Transition-Intervention (n = 14) and Baseline-Intervention farms (n = 17). A total of 63 farms (61.8% of recruited farms) stopped raising chickens and are not displayed in the graphs.

TABLE 2 | Descriptive data on AMU, mortality and bodyweight of chicken study flocks.

Status	Farms	No. flocks	No. weeks	AMU Mean (±SE)	Mortality Mean (±SE)	Birds at point of sale	
						Mean weight (g) (±SE)	Mean age (±SE)
Baseline	Baseline-Transition-Stop	9	179	160.0 (±44.2)	1.26 (±0.4)	1,590 (±40)	19.9 (±0.8)
	Baseline-Transition-Intervention	42	728	333.3 (±46.0)	1.43 (±0.2)	1,560 (±30)	17.5 (±0.3)
	Baseline-Intervention	45	823	352.3 (±48.4)	1.75 (±0.3)	1,560 (±30)	18.5 (±0.4)
	Baseline-Intervention (all)	87	1,551	343.4 (±33.5)	1.60 (±0.2)	1,560 (±20)	18.0 (±0.3)
	Baseline-Control	13	253	216.8 (±71.8)	1.17 (±0.2)	1,680 (±110)	19.5 (±0.7)
	Baseline-Stop	110	1,916	387.7 (±36.2)	3.18 (±0.3)	1,540 (±20)	18.3 (±0.3)
	All baseline	219	3,899	348.5 (±22.8)	2.33 (±0.2)	1,560 (±10)	18.4 (±0.2)
Transition	Baseline-Transition-Stop	4	74	316.1 (±107.4)	2.62 (±0.8)	1,540 (±70)	18.5 (±1.0)
	Baseline-Transition-Intervention	18	322	407.3 (±82.0)	1.78 (±0.4)	1,530 (±30)	17.9 (±0.5)
	All Transition	22	396	390.3 (±69.6)	1.94 (±0.3)	1,530 (±30)	18.0 (±0.5)
Intervention	Baseline-Transition-Intervention	37	648	191.3 (±35.6)	1.92 (±0.4)	1,620 (±40)	17.8 (±0.4)
	Baseline-Intervention	40	702	254.1 (±47.4)	1.39 (±0.4)	1,710 (±50)	17.7 (±0.4)
	All Intervention	77	1,350	223.9 (±30.0)	1.64 (±0.2)	1,670 (±30)	17.8 (±0.3)
Control	Baseline-Control	12	227	182.5 (±56.3)	1.29 (±0.3)	1,600 (±60)	18.9 (±0.9)
All		330	5,872	316.3 (±17.5)	2.11 (±0.1)	1,580 (±10)	18.2 (±0.2)

AMU: Expressed as weekly average No. of ADD_{kg} per 1,000 kg chicken-days. Mortality: Percent of chickens dying weekly. Bodyweight: Weight of chickens at slaughter-age.

TABLE 3 | Mixed regression models investigating the effectiveness of the intervention on AMU, mortality, and chicken bodyweight.

Models	Weekly ADD$^{\dagger}_{kg}$			Weekly mortality†††			Chicken bodyweight††† (unit = 100 g)		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value	β	95% CI	*p*-value
Intervention Arm									
Univariable									
Thap Muoi district (baseline = Cao Lanh)	0.68	0.37–1.26	0.220	1.60	1.13–2.26	0.007	0.73	−0.30 to 1.77	0.179
No. of restocked chickens (log)	0.55	0.36–0.85	0.007	1.34	1.02–1.47	0.032	−0.08	−0.78 to 0.65	0.820
Status (baseline = Baseline)									
Transition	0.77	0.30–2.01	0.598	1.38	0.76–2.52	0.294	−0.46	−1.49 to 0.58	0.390
Intervention	0.33	0.17–0.65	0.001	0.57	0.40–0.82	0.002	1.00	0.37 to 1.64	0.002
Multivariable 1									
Thap Muoi district (baseline = Cao Lanh)				1.61	1.14–2.29	0.007			
No. of restocked chickens (log)	0.55	0.36–0.85	0.007	1.39	1.07–1.81	0.015	−0.05	−0.72 to 0.65	0.884
Phase (baseline = Baseline)									
Transition	0.86	0.34–2.18	0.743	1.35	0.74–2.45	0.327	−0.45	−1.49 to 0.59	0.393
Intervention	0.34	0.18–0.66	0.002	0.60	0.41–0.86	0.005	1.00	0.37 to 1.64	0.002
Multivariable 2									
Thap Muoi district (baseline = Cao Lanh)				1.62	1.14–2.30	0.007			
No. of restocked chickens (log)	0.55	0.36–0.85	0.007	1.39	1.07–1.81	0.015	−0.06	−0.73 to 0.63	0.842
Phase (baseline=Baseline)									
Transition	0.86	0.34–2.18	0.743	1.35	0.74–2.45	0.327	−0.46	−1.48 to 0.60	0.404
First intervention cycle	0.46	0.20–1.05	0.066	0.57	0.36–0.92	0.022	0.77	−0.04 to 1.59	0.068
Subsequent intervention cycle	0.26	0.10–0.63	0.003	0.61	0.40–0.94	0.025	1.19	0.44 to 1.97	0.003
Control Arm									
Univariable									
Thap Muoi district (baseline = Cao Lanh)	0.95	0.15–6.13	0.958	1.88	0.49–7.32	0.360	1.49	−3.74 to 6.71	0.672
No. of restocked chickens (log)	0.19	0.04–0.87	0.033	0.08	0.04–0.17	<0.001	1.26	−2.01 to 4.49	0.471
Status (baseline = Baseline)									
Intervention calendar time	1.07	0.18–6.23	0.937	0.60	0.17–2.09	0.419	−0.02	−2.72 to 2.23	0.983
Multivariable 3									
Thap Muoi district (baseline = Cao Lanh)	0.64	0.09–1.73	0.666	0.53	0.22–1.27	0.156	2.33	−3.19 to 7.88	0.594
No. of restocked chickens (log)	0.15	0.03–0.87	0.034	0.07	0.04–0.15	<0.001	1.73	−1.65 to 4.65	0.383
Phase (baseline = Baseline)									
Intervention calendar time	1.42	0.24–8.44	0.696	0.81	0.38–1.74	0.591	0.14	−2.74 to 2.21	0.905

Multivariable 1 intercepts: † *−11.887 (SE = 1.303);* †† *−11.400 (SE = 0.794);* †††*16.093 (SE = 1.908). Multivariable 2 intercepts:* † *−11.897 (SE = 1.308);* †† *−11.400 (SE = 0.793);* ††† *16.093 (SE = 1.908). Multivariable 3 intercepts:* † *−5.135 (SE = 5.539);* †† *7.382 (SE = 2.297);* ††† *4.558 (SE = 12.129).*

(HR = 0.60; 95% CI 0.41–0.86; $p = 0.005$) and bodyweight (+100 g; 95% CI 37–164 g; $p = 0.002$). When the variable level "status = intervention" was replaced by two new variables (first, subsequent cycles), greater reductions in AMU were seen in the subsequent (HR = 0.26, $p = 0.003$) compared with the first cycle (HR = 0.46; $p = 0.066$), although the difference between both was not statistically significant ($p = 0.298$). Similarly, chicken bodyweight further increased during subsequent intervention cycles (+119 g per chicken sold, $p = 0.003$) compared with the first intervention cycle (+77 g, $p = 0.068$). However, there was no statistical significance in chicken bodyweight between first and subsequent intervention cycles ($p = 0.378$). Levels of mortality did not change between first and subsequent cycles ($p = 0.967$). There were no significant interactions between either "flock size" and "district" and "Status = intervention." There was no statistical difference in AMU and mortality between flocks using Product A, Product B or those given no additional product. However, flocks that were administered with either Product A and B had increased bodyweight compared with flocks not given any supplementary product (data not shown). Data from these flocks were kept in the final models after confirming that their removal did not change model coefficients to a large degree: AMU reduced from HR = 0.34 including them compared with HR = 0.33 when excluded. With regards to mortality, the removal of these observations resulted in HR = 0.53 compared with HR = 0.60 obtained with the whole dataset.

In the control arm, there were no significantly associations between "Status = intervention calendar time" and any of the three outcome variables in either univariable or multivariable models (all $p > 0.419$). After adjustment of flock size and

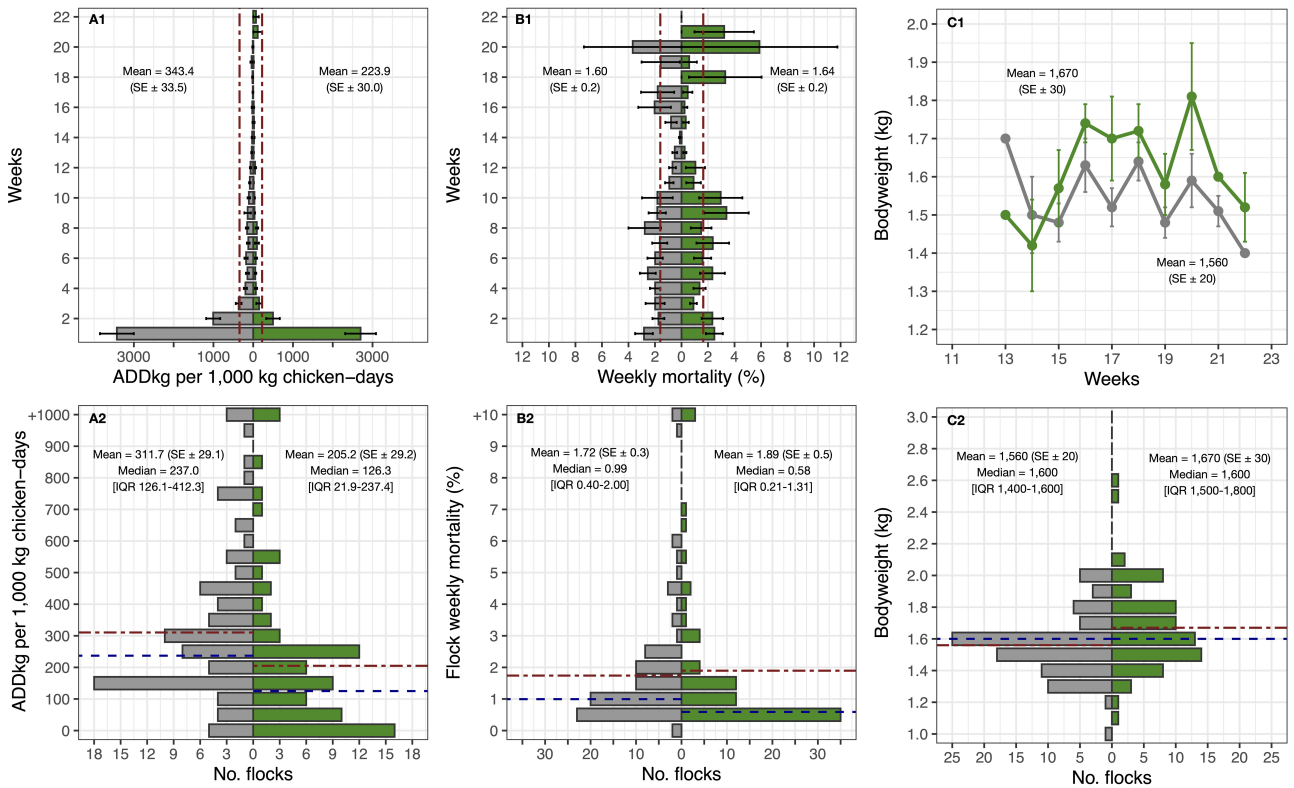

FIGURE 3 | Comparisons between AMU, mortality and bodyweight by weeks and flocks between baseline (in gray color) and intervention phase (in green color) in 31 eligible farms. Dot-dash red line: Mean. Dashed blue line: Median.

study district in multivariable models, estimates of AMU and bodyweight increased (+42% and +14 g, respectively) and mortality was reduced (−19%).

DISCUSSION

Through a locally delivered veterinary intervention, we achieved a 66% reduction in antimicrobials (quantified as daily doses) administered to small-scale commercial chicken flocks, alongside a reduction in mortality (−40%). In our crude (unadjusted) analyses AMU reductions were, however, modest (−35%), since our analysis implicitly adjust for week of use and most AMU took place during the early weeks (i.e., the brooding period). Similarly, the crude data indicated a slightly higher mortality during the intervention (+2.4%). However, the adjusted analysis indicated a ∼40% reduction in mortality, and weekly mortality was reduced in a majority (19/31, 61.3%) of farms. This discrepancy was explained by unusually high mortality in three intervention flocks.

Unlike other studies involving the delivery of a uniform treatment (i.e., vaccination) (25), our intervention consisted of providing farmers with veterinary advice. The nature of this advice was variable across farms, and was based on specific observations and information collected by project veterinarians from their flocks. This advice included measures to improve flock health and productivity, whilst emphasizing the message that "antimicrobials should not be admnistered to healthy chickens."

In addition to providing antimicrobial replacement products, the main advice given to farmers focused on biosecurity, cleaning and disinfection, vaccination, litter management, and administration of medicines (including antimicrobials, antiparasitic drugs and other health-enhancing products). The detail of this advice provided and its uptake will be presented elsewhere. The advice provided was based on a persuasive, rather than a restrictive advice. We believe this approach is likely to be more sustainable in the mid-to-long term (26). A similar holistic approach was adopted on a study on pig farms in Belgium, resulting in 52% AMU reduction in pigs raised from birth to slaughter, and by 32% among breeding animals; furthermore, the study resulted in additional productivity gains (20). Similarly, a study conducted in four European Union (EU) countries reported AMU reductions of 3 and 54% in fattening and weaned pigs, respectively, following improvements of herd management practices (19). However, reductions in AMU were not seen in breeding pigs, and the authors attributed it to the concurrent incursion of Porcine Epidemic Diarrhea (PED) in Germany. A study in 20 industrial-scale broiler farms in Europe using a holistic approach resulted in 20% reductions in levels of AMU and 14% increase in gross margins (22).

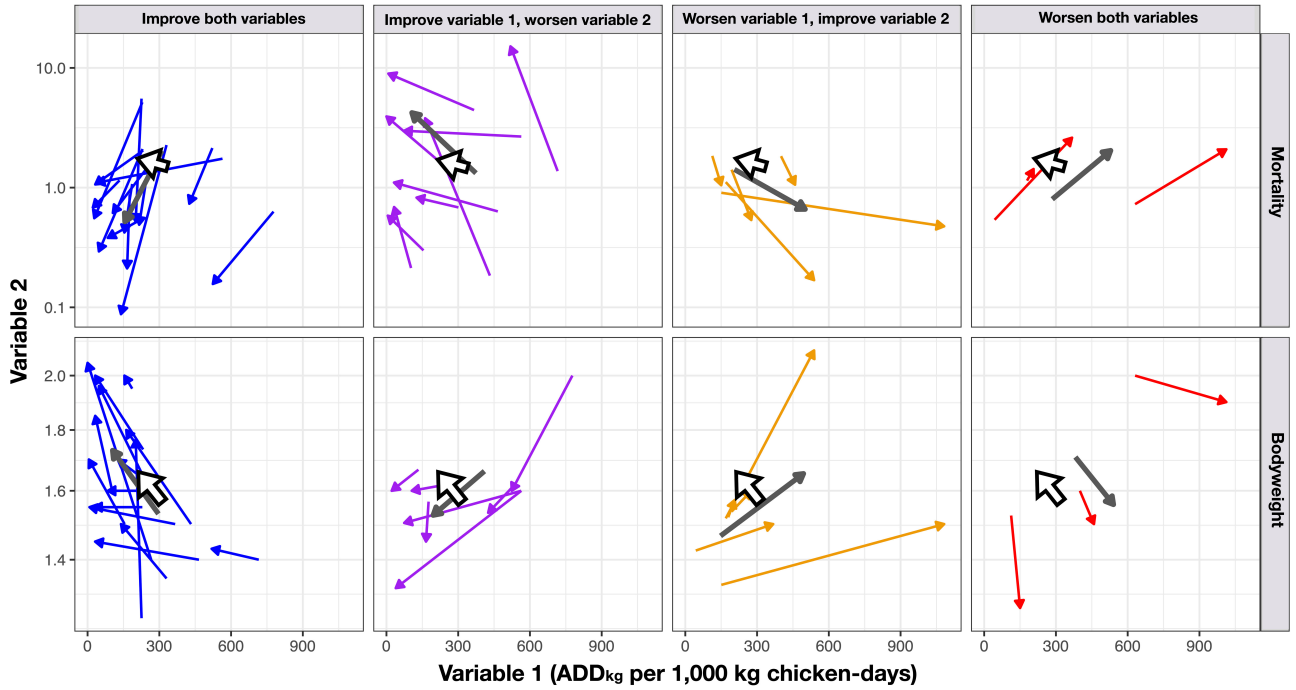

FIGURE 4 | Bi-dimensional arrow charts showing crude (unadjusted) changes between the baseline and the intervention phases with regards to Variable 1 (No. ADDkg per 1,000 kg chicken-days) and Variable 2 (mortality and bodyweight). The arrow indicates the direction of change. Large gray arrows indicate summary result for each pair of variables. Large white arrows indicate overall results. The log10-transformed scale for easier visualization.

After consultation with participating farmers during the baseline phase, we were compelled to modify our original protocol by offering selected health-enhancing, antimicrobial replacement products (27) to chicks during the brooding phase. This aimed at allaying the farmers' anxiety about reducing or eliminating antimicrobials during this critical phase of production. Administration of antimicrobials during the brooding phase is standard practice and many antimicrobial-containing commercial formulations are marketed as "brooding medicine" (13). Similarly, many of our study farmers expressed their opposition about changing the feed and therefore we consolidated the two intervention arms into only one arm. Often the advice provided by project veterinarians to farmers was overrun by that given at local veterinary drug shops. Farmers often visit these shops to buy animal feed and other supplies (17). In addition, the antimicrobial product labels often include indications for prophylactic use at a lower dose (28).

Small-scale commercial chicken production using native breeds is widespread in the Mekong Delta of Vietnam, and often represents an upgrade from backyard production. The popularity of this system resides in the preference of the Vietnamese consumer for meat of long-cycle native birds. Native chicken meat reaches a considerably higher price compared with broiler meat (29). However native chickens (and their crosses) are slow growing (>4 months), and preventing disease over such a prolonged period requires sustained efforts (13).

In our study, the identification and enrollment of study farms was challenging due to the fluidity of this type of production system, with many households setting up chicken farms as well as stopping raising chickens altogether. Because of this, a large number of farms did not remain in business over the extended duration of the study. Indeed, flock mortality was an important predictor for farmers giving up raising chickens (data not shown) and a large fraction of our study farms (61.8%) had gone out of business even before the start of the planned intervention phase.

In addition to their previous experience with disease, farmers may start or stop raising chickens depending on circumstances, such as market price of day-olds, commercial feed and poultry meat, income from the sale of the previous flocks or other rural activities. Furthermore, many farmers raised one cycle per year, but not necessarily every year. This was reflected in the lack of experience in chicken husbandry of many farmers (and farm workers). This represents a hurdle for the implementation of correct management practices. This contrasts with a recent study in Belgium, where pig farmers had on average 22.6 years of experience (20). In this context, often antimicrobials are used as replacement of other, most costly, but demanding husbandry practices (14). The incursion of African Swine Fever (ASF) in Vietnam in January 2019 and its spread within the country (30) coincided with the intervention phase in this study. This may have exerted additional pressures over our study farmers. During this time, many farms in ASF-affected provinces switched to chicken production, resulting in increased market availability of low-cost chicken meat, therefore reducing the value of chicken production in our area.

The changes to the initial study design are a testament to the challenges of conducting intervention studies in small-scale farming systems. Initially, we planned to allocate one third of all recruited farms to the control arm in order to measure any environmental influences on AMU, for example, due to public engagement initiatives (television campaigns, work in schools, etc.) that took place in the province under the umbrella of this project. Exposure to these may have inadvertently had an influence on the farmers' decision on AMU beyond the intervention. Given the high number of farms that stopped chicken production, we opted for reducing the size of the control arm to a minimum of four, thus reducing the statistical power of any analysis in that group. However, the descriptive data from this small control group suggests no change between baseline and intervention, and gives additional validity of the observed findings.

The study demonstrates that reducing current high levels of AMU through the provision of veterinary advice is achievable in the Vietnamese small-scale commercial farming context. There was an indication that farmers responded to the advice given. Many farmers, especially the larger ones may even be willing to pay for such a service, since labor costs in Vietnam are relatively low (∼25 USD for a 2-h visit). We believe that results and lessons from this study can be adapted other LMICs where small-scale animal production systems are common. However, the usefulness of this approach for with regards to intensive farming systems requires further investigation, since such systems already have their own technical advisory services. Sustainable, long-term reductions in AMU could be reached if links between veterinarians/animal health workers and farmers are built and reinforced. Supplementation with health-enhancing products may be beneficial, but this needs to be further explored. We propose to develop a business case for an advisory service targeting the main livestock-producing regions in the country (Mekong River Delta, Southeast, Central region, Red River Delta), with the value proposition that healthy livestock means profitable businesses.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Oxford Tropical Research Ethics Committee (OxTREC). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Oxford Tropical Research Ethics Committee (OxTREC). Written informed consent was obtained from the owners for the participation of their animals in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. The study has been granted ethics approval by the Oxford Tropical Research Ethics Committee (OXTREC) (Ref. 5121/16).

AUTHOR CONTRIBUTIONS

JC-M, JR, and NC conceived and designed study. DP, NC, and DT conducted field survey. BK and VH designed and aided data collection. HT, LY, NM, and ES aided intervention packages. DP, JC-M, NC, and DT contributed to data analyses. DP, NC, JC-M, PP, JR, and GT contributed to writing up and editing the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.612993/full#supplementary-material

Supplementary Figure 1 | Study area in the province of Dong Thap, Mekong Delta of Vietnam.

Supplementary Figure 2 | Correlation between AMU, mortality and bodyweight by week (left block) and by flock (right block). The data have been log-transformed scale for easier visualization.

Supplementary Table 1 | Descriptive characteristics of chicken farms by total of farms, flocks, and weeks.

Supplementary Data 1 | Raw data for calculation of ADD_{kg} per 1,000 kg chicken-days.

Supplementary Data 2 | Raw data on AMU, mortality, and bodyweight.

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